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CLINICAL AND EXPERIMENTAL

THE CLASSIFICATION OF ALLERGIC DISEASES*
WITH REFERENCE TO DIAGNOSIS AND TREATMENT

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DURING the past twenty years we have witnessed the rapid growth of a new medical specialty which is based upon a newly recognized principle of the causation of disease. This principle lies in the surprising paradox that antibodies, which had previously been known to medicine chiefly as protectors against disease, often represent the actual cause of disease.

This concept was formulated on the basis of animal experiments in anaphylaxis long before the peculiar antibodies of the corresponding allergic conditions in the human being were discovered.

These peculiar human antibodies are not demonstrable in all of the allergies; not in any of those exhibited toward the alkaloids such as quinine, chemicals, such as, nickel and arsenic and the excitants of what was formerly referred to as dermatitis venenata, but is now known as contact dermatitis. They are also often missing in cases of urticaria or angioneurotic edema especially those in which there is no associated involvement of the mucous membranes (bronchial, nasal, gastrointestinal). They are however, regularly present in hay fever, in bronchial asthma when the asthma is caused by a specific antigenic excitant, in the inherited eczema, which is associated by heredity with hay fever and asthma, in gastrointestinal allergy and possibly in some other clinical expressions of allergy. They have been given the name "atopic reagins" or skin sensitizing antibodies.

When the reagin-containing serum of a hypersensitive person is injected into the skin of a normal person, the injected site soon becomes sensitive to the same substances to which the patient is sensitive and this passive skin sensitiveness persists for several weeks.

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Evidently if the allergic person's serum is capable of sensitizing another person's skin his own skin must be constantly sensitized by the blood passing through it; consequently, if the sensitive person's skin fails to react to a certain material and if the test was properly applied, then it would seem to be useless to look for reagins in his blood with the method of passive skin sensitization which I have just described.

Practically, however, this is not the case, since Walzer finds that in some instances, in which the reagin content of the patient's serum is so low that there is only a slight though significant difference between the reactions in the passively sensitized site and the control (see below under Indirect Test), the reactions in the patient's skin are too slight to be recognized as specific.

For all allergic conditions in which reagins are participating, there are two kinds of tests which are used in searching for a specific excitant; these are the cutaneous tests and the ophthalmic test.

CUTANEOUS TESTS

It seems probable that the controversy over the relative merits of the scratch method and the intracutaneous method of applying the skin test will end in a draw, because the two methods have been found to yield the same results provided they are properly applied with suitable extracts by competent observers.

The quantitative comparative tests of Fineman[1] showed that if sufficiently strong fluid extracts are used with the scratch technic, the results are the same as those obtained with the intracutaneous method with the more dilute extracts.

With this principle in mind, I have prepared concentrated extracts and have preserved these with glycerin for use with the scratch method. In many comparative tests with the two methods the results with all of the extracts thus far examined have been diagnostically the same.

On account of this equality of the two methods as to the results the choice between them will be made with regard to their differences in other respects. Let us examine these differences.

1. Undoubtedly, fewer constitutional effects are produced with the scratch technic than with the intracutaneous injection method.

2. There is usually less likelihood of a nonspecific reaction with the scratch method properly performed than with the intracutaneous method, which is more difficult to carry out properly.

3. The intracutaneous method requires much more paraphernalia, and a greater preparation of them than is needed for the simple scratch technic.

4. The proper interpretation of the reactions obtained with the intracutaneous technic calls for decidedly more experience than is usually necessary for the reading of the scratch reactions.

On account of all of these four considerations the scratch method will usually be preferred by physicians who see only few cases of allergic disease and whose patients, for one reason or another, are not able to consult an experienced allergist.*

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[1]Fineman, A. H.: J. Immunol. **11**: 465, 1926.

*The allergic specialist is permanently established in medicine. The proper handling of the allergies requires much more than a knowledge of skin testing. But as with other medical specialties it is important that all medical men be able to make a preliminary diagnosis.

On the other hand, the intracutaneous technic has certain important advantages over the scratch method: it is cleaner; for the large scale testing by the specialist it is much quicker; it is not necessary to keep the patient's arm in a fixed position; the results can be read in much shorter time (five to ten minutes); there is less scarring of the skin. Furthermore, the intracutaneous method allows testing with different dilutions of the extracts; and there is also the advantage that the same extract can be used for treatment as well as for testing.

Finally, the intracutaneous technic is indispensable for the indirect method of Walzer, which I shall describe presently, and for testing with "autogenous" extracts of dust collected from the patient's living or work room, which are not strong enough for scratch testing.

The most disturbing effect of the nonspecific skin reaction with the intracutaneous technic is, no doubt, seen in the tests for horse serum sensitivity that are made previous to the administration of therapeutic horse serum. If the patient has previously had even a small injection of the serum, as in toxin-antitoxin immunization, he may exhibit an acquired skin sensitivity which does not indicate a constitutional sensitiveness and which should not be taken as a contraindication to the administration of the serum.

In such a situation, the ophthalmic test is a much more reliable one for the existence of the dangerous atopic hypersensitiveness to serum.

THE OPHTHALMIC TEST

This test is in some disrepute, possibly on account of the serious damage to the eye which has been reported when the test was applied with tuberculin in tuberculin sensitive persons.

The tuberculin reaction is inflammatory and appears after some hours; it cannot be controlled by any known means. The atopic ophthalmic reaction, on the contrary, is limited to congestion and edema; it appears within a few minutes, and it can usually be immediately controlled with the local application of epinephrin.

In testing the eye for horse serum sensitivity one drop of a 1-10 dilution of horse serum (Park uses the undiluted serum) may be dropped in the lower sac and if there is no congestion or itching after ten minutes the presence of atopic sensitivity to the serum can be excluded and the serum can be administered.

The tests that have been described are all tests of reaginic sensitivity; that is, the positive reactions usually depend on the presence in the blood of the subject of the peculiar human antibodies known as atopic reagins. This circumstance gave the foundation upon which the indirect method of skin testing, which I am about to describe, was developed by Matthew Walzer.

THE INDIRECT TEST

In some circumstances, it is impossible or inadvisable to test the subject directly. In such case the indirect method of Walzer can be used with great advantage. Walzer makes use of the Prausnitz-Kuestner technic as follows:

One-twentieth of a cubic centimeter of the patient's sterile serum is injected intracutaneously in 36 sites in the upper arms of a substitute, usually a relative, who should not be atopic. After three to seven days, these sensitized sites are tested by the intracutaneous injection of the various extracts and at the same time control injections of the same extracts are made in unsensitized sites. If the reaction in the sensitized site is in any degree greater than that in the unsensitized skin this result is taken as an indication of a corresponding sensitivity in the patient; it shows that the patient's blood contains reagins specific for the material used in the test.

This test is plainly superior to any mode of direct test excepting the clinical test, which is carried out by exposing the patient to natural contact with the material as by inhalation or ingestion. Actually the indirect test is the ultimate criterion of the specificity of a cutaneous or ophthalmic reaction.

The control is practically perfect and even slight differences in the reaction in the sensitized and unsensitized skin are diagnostically significant. Walzer states, for example, that if the test in the unsensitized skin produces no wheal or erythema whereas the sensitized site shows erythema without wheal formation this result can be accepted as a specific reaction.

INDICATIONS FOR THE USE OF INDIRECT TESTING

Some of the indications given by Walzer are:

1. Abnormal skin conditions of the patient such as, chronic atopic eczema, ichthyotic skins, urticaria, dermographism, and the presence of contagious skin infection (impetigo).

2. Constant severe asthma necessitating the continual use of epinephrin. If the patient is under the influence of epinephrin, a cutaneous reactivity may be suppressed by the drug.

3. Extreme sensitivity where constitutional reactions might result from the direct tests.

4. The presence of other severe illness.

5. Antipathy to direct test on the part of the patient or his relatives.

6. Inability of the patient to come to the physician's office.

7. The desire to test the genuineness (specificity) of an unusual number of positive reactions elicited by direct testing.

The indirect test has been suggested also by J. Alexander Clarke as a test of reaginic horse serum sensitivity previous to the administration of therapeutic horse serum.

The allergic diseases that are due to a reaginic sensitivity are all subject to a Mendelian hereditary influence, which determines the percentage incidence of this kind of allergy in the offspring, the age of onset of the symptoms, and the clinical form of the allergy.

CONTACT DERMATITIS

There is another form of allergic disease which is not subject to a special hereditary influence and in which no antibodies have yet been demonstrated. This form has been called "dermatitis venenata" but is now known by the better name "contact dermatitis."

The list of the known excitants of "contact dermatitis" is large and it is still growing. Beside the well known causes of "dermatitis venenata," poison ivy, poison oak, poison sumac, primrose and others, there are certain dyes used for coloring furs such as ursol, the metals arsenic, nickel, lead and mercury, and a host of miscellaneous materials such as printer's ink, iodoform, adhesive plaster, formalin, furniture polish, and others. There are also many reported instances of dermatitis due to local contact with various cereals, vegetables and pollens.

A recent study of ragweed pollen dermatitis has shown that the excitant of the condition is not the exciting cause of hay fever in the pollen but the oil of the pollen. This result suggests that contact dermatitis caused by cereals and vegetables may be due to the oils in these materials. We are continuing the investigation of this question and are in need of clinical material of this kind. We should, therefore, be most thankful if any of you who see dermatitis that seems to be caused by direct contact with some vegetable or cereal would test and possibly treat such cases with the oil of the respective material which I should be glad to send you upon request.

The test with the excitants of contact dermatitis is not made by injection or with the scratch technic. Cooke has found that the intracutaneous injection of poison ivy extract fails to produce either a wheal or the typical lesion of ivy sensitiveness.

The cutaneous test for contact dermatitis is made by applying the suspected material to the uninjured surface of the skin and leaving it there for one to five days (J. Jadassohn).

The circumstances under which a person may have natural contact with the excitant of contact dermatitis differ greatly with the different substances; it is very rarely if ever that this lesion follows the ingestion of the excitant, although such excitants frequently occur in food materials (cereals and vegetables); contact with particles of the excitant which are carried by the wind often takes place, as in pollen dermatitis and no doubt also in ivy sensitivity; perhaps the most frequent manner of contact is that had by handling or accidentally touching the material in which is the exciting substance.

It is evident from the foregoing that many a sensitive person may not be known as such because the natural contact which he has with the particular material is not sufficient to induce a reaction; in such cases the existence of the sensitivity must be determined by testing.

The incidence of the sensitivity of contact dermatitis as determined by deliberate testing differs with the different materials. According to Spain, about 60 per cent of persons over three years of age in New York City exhibit sensitivity to poison ivy by test; and H. W. Straus was able to sensitize over 70 per cent of newborn by application of an ivy extract to the skin. Brown, Milford and Coca report that 15 per cent of all persons are sensitive to the oil of ragweed pollen. Bloch states that 100 per cent of human beings can be sensitized with an extract of primrose.

These high percentages compared with that of the inherited group, about 7 according to Spain and Cooke, indicate that contact dermatitis is not subject to the hereditary influence which controls the hypersensitiveness of the asthma,

hay fever, eczema group; and this conclusion is confirmed by the personal observation that the incidence of contact dermatitis is the same among asthmatics or hay fever subjects as it is among persons free from these conditions.

In the light of the foregoing discussion of the classification of allergic diseases, it is instructive to consider the differences in the etiology, diagnosis, and treatment of the two forms of allergic disease of the skin, the hereditary eczema and the nonhereditary contact dermatitis.

For convenience, I have arranged these differences in tabular form in Table I.

TABLE I

ATOPIC ECZEMA	CONTACT DERMATITIS
HEREDITARY	NONHEREDITARY
Hereditary abnormality of skin	No hereditary abnormality of skin
Frequently mediated with reagins	No reagins
Water-soluble antigenic excitant	Nonantigenic excitant often soluble in fat solvents
Acquired by ingestion	Acquired by surface contact
Skin test by injection or scratch	Skin test by surface application

The specific treatment of contact dermatitis is similar in principle to that used in the atopic conditions (asthma and hay fever); that is, the injection of the specific excitant. It has been resorted to chiefly in cases of sensitiveness to the water insoluble excitant of poison ivy and the most satisfactory menstruum has been found to be sterile almond oil. Two or three injections of this preparation seem to be sufficient in most cases to establish the desired tolerance.

Similar results are being obtained in pollen dermatitis with pollen oil dissolved in the same menstruum.

In conclusion, it may be well to recapitulate the chief points of this paper as follows: leaving out of consideration serum disease, which occurs under artificial conditions, there are two distinct categories of allergic disease; namely the atopic diseases of asthma, hay fever, eczema and others and, contact dermatitis. These two categories differ in their etiology, in their mechanism, and in the methods employed in the identification of their respective excitants. The former require the use of aqueous extracts with the intracutaneous or scratch technic; the latter require surface testing with extracts of nonantigens many of which are soluble only in fat solvents.

It is evidently important, in order that the proper material and mode of testing be employed, that the physician recognize in the particular case before him to which of these two categories it belongs.

A-V DISSOCIATION*

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THE interpretation of curves showing A-V dissociation presents many difficulties which have not been thoroughly evaluated in the past. Furthermore several closely related questions are involved, such as reciprocal beating of the heart, the supernormal phase, prolongation of the P-R conduction interval, and variation of the P-P intervals in A-V block. The present report is made in an endeavor to clarify the analysis of such curves. In the following presentation I have attempted to treat the subject of A-V dissociation in logical order, the facts presented being derived from the study of original curves which were correlated with an analysis of previous reports.

A-V dissociation denotes the condition in which the auricles and the ventricles beat independently of each other, that is, the auricles and the ventricles are controlled by different pacemakers. If no ventricular beat is a response to the pacemaker controlling the auricles, and vice versa, then there is complete dissociation; if some but not all of the ventricular beats are in response to the same pacemaker controlling the auricles, or vice versa, then there is incomplete dissociation or dissociation with interference.

The best known and most common example of complete A-V dissociation is in complete A-V block without any other disturbance; the auricles are controlled by the S-A node, the ventricles by a pacemaker in the A-V node or in the crus commune, and none of the impulses pass from the auricles to the ventricles or vice versa. A-V dissociation and complete A-V block are, however, by no means synonymous; although complete A-V block is always accompanied by complete A-V dissociation, the converse is by no means true. It is necessary to distinguish also between primary or essential A-V block and secondary or adventitious A-V block. By primary A-V block is meant A-V block due to prolongation of the refractory period of the A-V node or crus commune, such as in the blocking of a normal auricular impulse in normal sinus rhythm. By adventitious A-V block is meant A-V block due not to prolongation of the refractory period but to the fact that the impulse reaches the A-V node or crus commune during its normal cyclic refractory period, such as in the blocking of a very early auricular extrasystole,[28] and in auricular fibrillation and flutter. Practically all ventricular extrasystoles are blocked from the auricles, that is, such an extrasystole is rarely conducted back to the auricles.† The same is true in most cases of ventricular paroxysmal tachycardia, so that the auricles beat in response to their own pacemaker. Consequently there is momentary A-V dissociation in ventricular extrasystoles and in very early auricular extrasystoles, and complete A-V dissociation

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†When it is conducted backward it is a retrograde ventricular extrasystole.

in ventricular paroxysmal tachycardia. Aside from these obvious instances the study of A-V dissociation may be divided into two parts, without and with primary A-V block.

A-V DISSOCIATION WITHOUT PRIMARY A-V BLOCK

In this condition the auricles are controlled by the S-A node and the ventricles by the A-V node. If we let A and V represent the auricular and ventricular rates, respectively,* then it is obvious that $A \leqq V$; for if A were greater than V the auricular impulses would be conducted to the ventricles since there is no primary A-V block.

The principle of the mechanism is best illustrated by the diagram (Fig. 1) of an ideal case. Let A and V both be constant, A = 67, V = 75, and let A_o and V_o be simultaneous. There is a regular sequence A_o, A_1, ...of auricular beats.

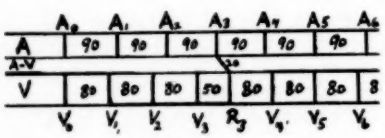

Fig. 1

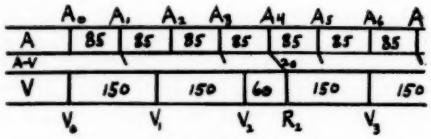

Fig. 2

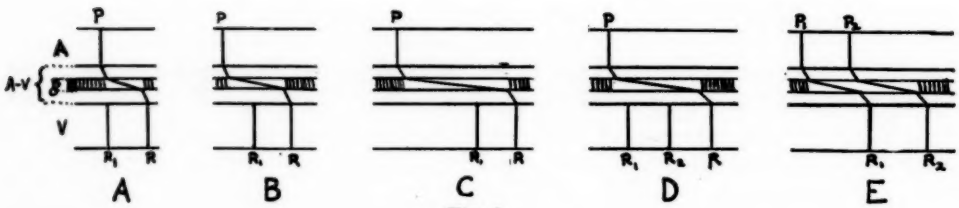

Fig. 3.

Fig. 1.—Diagram of interference-dissociation; intervals in hundredths of a second.
Fig. 2.—Diagram of dissociation with interference in primary 2:1 A-V block.
Fig. 3.—Diagram of five types of skip-conduction. B is a region of almost complete forward and complete retrograde block. O is just below B.

The impulse A_1 is not conducted because the recovery period $V_1A_1 = 10$ is too short.[28] Let the minimum recovery period for conduction be 25; then A_2 is not conducted, but A_3 is conducted because its recovery period is $V_3A_3 = 30$, which is above the minimum. Let the conduction time of A_3 be 20; there thus results a conducted beat R with an interval of coupling $V_3R_3 = 50$. The passage of the impulse from A_3 through the A-V node annihilates the partially formed nodal impulse, so that R_3V_4 is again 80. The recovery period $R_3A_4 = 70$, so that A_4 would be conducted did not V_4 follow A_4 too closely; A_5 and V_5 are again simultaneous, so that if A and V remain constant and if there is no change in A-V conductivity this process will continue in cycles, and every sixth ventricular beat will be conducted from the auricles, with a fixed coupling interval of 50.

In general, let a and v be the AA and VV intervals, $a > v$, and A_o and V_o be simultaneous. Now the recovery period of A_k is $V_kA_k = k\ (a\text{-}v)$; this increases until it is at least as big as the minimum recovery period, since there is no primary block, so that finally there is a conducted beat. Let A_m be the first conducted

*These terms A and V will be used throughout.

beat; its recovery period is m $(a\text{-}v)$; let its conduction time be $c = (n\text{-}m)$ $(a\text{-}v)$. Then the interval of coupling is $i = n$ $(a\text{-}v)$. Thus $na = nv + i$ and $ma + c = mv + i$, so that A_n and V_n are simultaneous; consequently every nth auricular beat is conducted and there are repeated cycles of n nodal ventricular beats followed by one conducted ventricular beat with fixed coupling. Here there is complete A-V dissociation except for the periodically conducted impulses; because the conducted impulse annihilates the partially formed nodal impulse it interferes with the nodal rhythm and shifts it; for this reason this condition, first described by Mobitz,[15, 16, 17] was called interference-dissociation. We note that $i = c + m$ $(a\text{-}v)$; the interval of coupling is at least the sum of the conduction time and the minimum recovery period. Normally the minimum conduction time is 12 to 20, and requires a recovery period of about 30, so that the sum of these two is 42 to 50; as the recovery period decreases the required conduction time increases without much change in their sum;[28] hence the coupling interval i has a relatively long minimum, say 40 to 50; in fact, the minimum of the coupling interval varies as the absolutely refractory period of the A-V node plus the conduction time from the A-V node to the ventricles.

If we depart from the ideal case we find the principle of the mechanism unaltered. Slight sinus or nodal arrhythmia changes the sequence of events, so that the intervals a, v, c, i are not constant but vary slightly. Similarly, changes in tonus of the vagi and sympathetics alter a, v, c, so that again the intervals vary. If we do not assume A_o and V_o simultaneous the numerical relations are altered. The result is that there is no exact periodicity of cycles, but there is still dissociation with occasional interference.

The same mechanism may give rise to complete A-V dissociation; for suppose $A = V$ and A_o precedes or follows V_o by a short interval, for example 10; then A_m precedes or follows V_m by 10 and is not conducted, so that the dissociation is complete. This, of course, occurs very rarely; it is illustrated in Leads I and II of Fig. 11 which will be discussed later. As stated before, we must have $A \leqq V$, whereas normally $A > V$. We may have $A \leqq V$ because of auricular bradycardia (which may be due to sinus bradycardia or sinoauricular block) or because of nodal tachycardia or because A and V are both increased or decreased but in different amounts. We present here two illustrative cases of interference-dissociation; the first is due to sinus bradycardia ($A = 45$, $V = 65$) and the second to nodal tachycardia ($A = 96$, $V = 99$).

CASE 1.—A curve taken before treatment shows sinus arrhythmia, rate 88, P-R = 0.16 second. Fig. 4 was taken after the patient had received 60 minims daily of tincture of digitalis for eight weeks; Fig. 5 was taken the same day as Fig. 4 and five minutes after subcutaneous injection of one-fiftieth grain of atropin sulphate. Then digitalis was stopped, and nine days later an electrocardiogram showed sinus rhythm, rate 86, P-R = 0.20 second.

In Figs. 4 and 5 are shown the time intervals in hundredths of a second; the oblique lines merely show the P-R and R-P intervals without connoting conduction. This method of analysis has the advantage of showing all the time intervals without committing itself to any particular interpretation.

In Lead I of Fig. 4 the P-waves are fairly regular, with an average P-P of

134,* so that A = 45. The R-R intervals, except the second, fourth, seventh and ninth, are nearly constant, 92 to 95, so that V = 65. The four shortened R-R intervals are due to conducted beats, as indicated by heavy oblique lines with arrows.

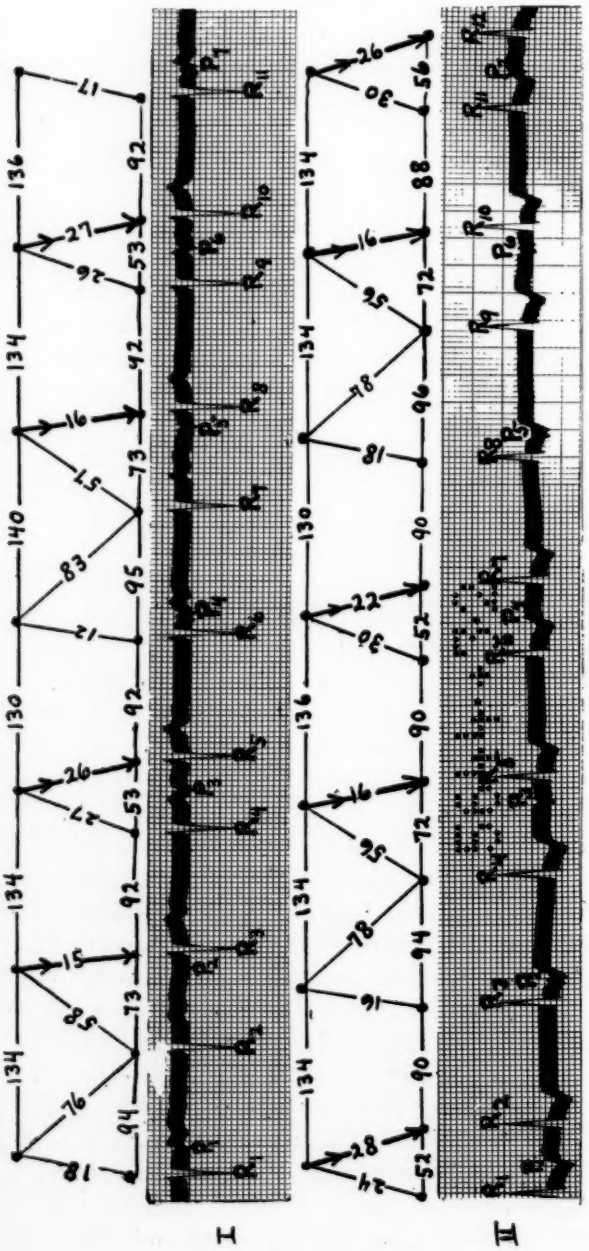

Fig. 4

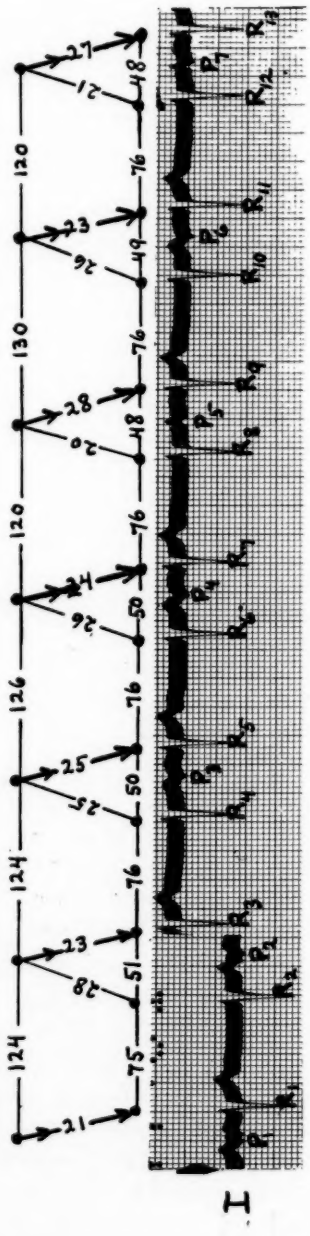

Fig. 5

Fig. 4.—Case 1. After eight weeks on digitalis, 60 minims daily, Leads I, II. Auricular bradycardia of 45. Adventitious A-V block, interference-dissociation.
Fig. 5.—Case 1. Same day as Fig. 2 but five minutes after injection of atropin. No A-V block at all; interference-dissociation; bigeminus due to escaped beats.

In Table I are tabulated the R-P intervals of all leads of Fig. 4 in decreasing order, and below them the corresponding P-R intervals of the next auricular impulse, using a dash (—) for absence of conduction. It is seen that P-R increases as R-P

*In hundredths of seconds.

decreases, as is to be expected.[28] The auricular impulses are conducted whenever they happen to come long enough after the preceding R so that the A-V node is no longer absolutely refractory when they reach it. The result in this case is 3:2 partial A-V block,* but this block is purely adventitious, as shown by the normal P-R of 16 when the recovery period is long enough. In Lead I the beats R_2, R_4, R_6, R_7, R_9, R_{11} are escaped nodal beats. In Fig. 5 all the P-waves are conducted to the ventricles; there is not even any adventitious block; but due to the auricular bradycardia every second ventricular beat is an escaped nodal beat.

TABLE I

R-P	60	58	57	57	56	56	30	30	27	26	26	26	24	<18 =
P-R	16	15	16	16	16	16	22	26	26	27	28	28	28	–

The following explanation of this case is offered. In Fig. 4 digitalis has produced an auricular bradycardia, without any effect on the A-V conduction time. Atropin had very little effect on the auricular rate, merely increasing it from 44 to 48; it also had very little effect on the A-V conduction time (which was already normal); the change from Fig. 4 to Fig. 5 is due entirely to the increased rate of the nodal rhythm. After nine days without digitalis the mechanism returned to normal, so that the arrhythmia was surely due to digitalis, the main action of which was to produce auricular bradycardia.

CASE 2.—The curve of Fig. 6 was taken after the patient had received digitalis for some time. There is a slight sinus arrhythmia, A = 96; except for four R-R intervals the ventricular rate is nearly constant, V = 99. The short R-R intervals are due to conducted beats as indicated. All the other P waves are blocked because they happen to come either too early or too late: in Lead I, P_6 to P_{10} come too early, R-P $\leqq$ 7, and they are adventitiously blocked; P_1 to P_5 come too late, so that the next R come before they have time to be conducted. For similar reasons there is no retrograde conduction. There is no primary block in this case. After three days without digitalis there was sinus rhythm with normal P-R intervals, so that the arrhythmia was certainly due to digitalis, which acted by producing nodal tachycardia.

CLINICAL DISCUSSION

This type of interference-dissociation is certainly uncommon, as shown by its incidence only twice in several thousand electrocardiograms observed. It is especially likely to occur in digitalized hearts. Without the electrocardiogram the diagnosis is practically not possible; it is, however, sometimes important. For example, in Case 1 the pulse or the apex beat at the time Fig. 5 was taken would very likely give the impression of pulsus bigeminus with auricular bradycardia and ventricular extrasystoles due to digitalis, and interpreted as a danger signal of severe digitalis intoxication. With the electrocardiogram the diagnosis is not difficult though it is usually missed. Fig. 6 was published as a case of complete A-V block, and the short couplings were left unexplained.[4] Fig. 5 is the type of

*I. e., every third auricular impulse fails to be conducted to the ventricles.

dissociation; bigeminus due to escaped beats. ... five minutes after injection of atropin. No A-V block at all; interference-

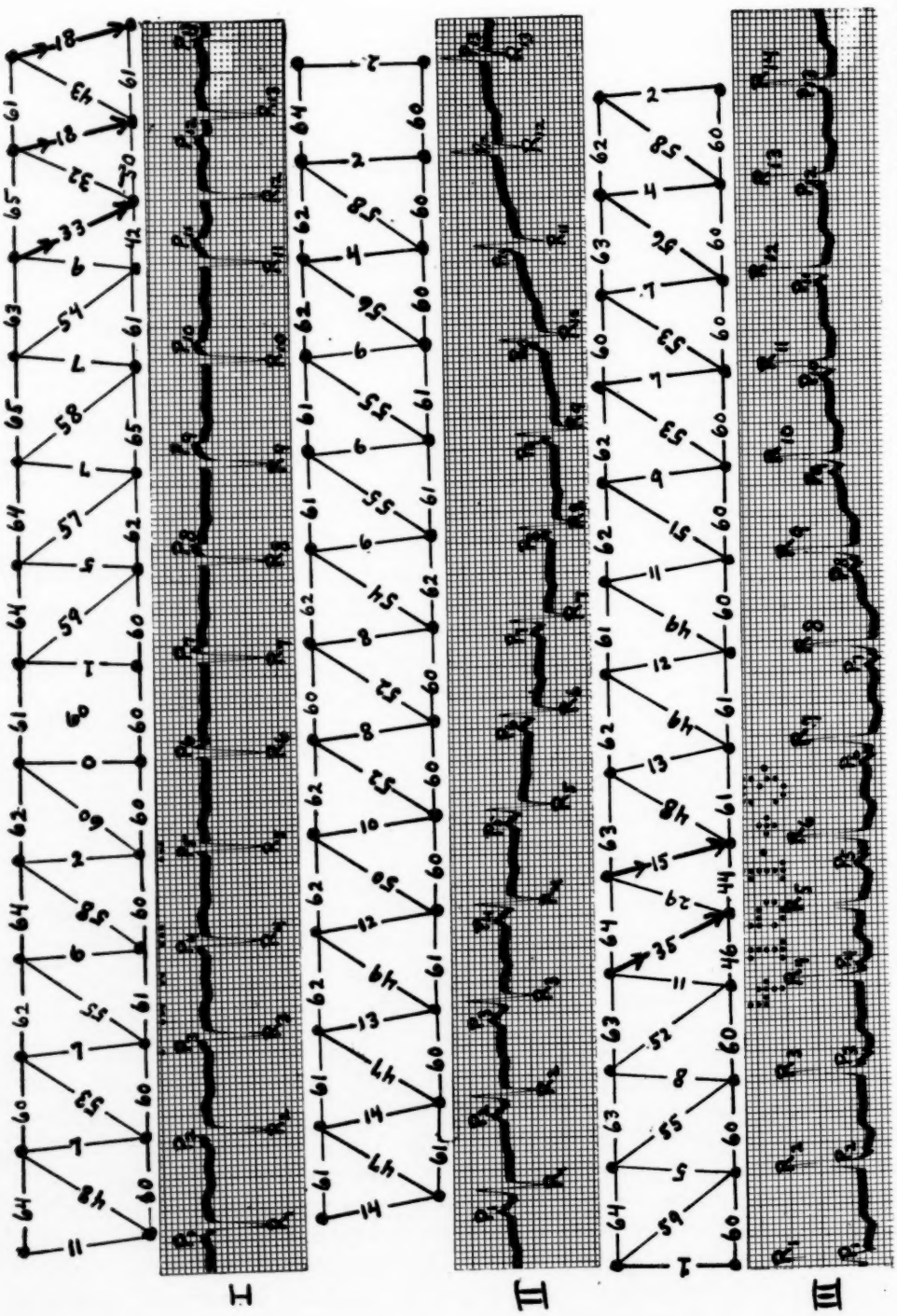

Fig. 6.—Case 2. Sinus rhythm, auricular tachycardia of 96, nodal tachycardia of 99. No primary A-V block; interference-dissociation, almost complete.

curve usually misinterpreted as reciprocal beating of the heart. In general, it may be said that in a regular series of supraventricular complexes interrupted by shorter R-R intervals dissociation with interference should be considered, and is especially likely to occur during digitalis administration.

Reciprocal Beating of the Heart.—Reciprocal beating of the heart is the condition in which an A-V nodal impulse is conducted back to the auricles, causing their contraction, and then this auricular impulse is conducted forward again to the ventricles and causes them to contract. A priori such an occurrence seems very unlikely indeed, and only a clear-cut example should be accepted as evidence for its occurrence. I have studied a number of cases reported in the literature as reciprocal beating;[3, 5, 6, 7, 8, 24, 25, 27] the reproductions of many of these curves are too indistinct for careful analysis. Before the mechanism can be called reciprocal beating it must be demonstrated that the P-wave between the coupled R-waves is actually retrograde, for which it is by no means sufficient that P be negative or deformed (see discussion of Fig. 11). Many of the reported curves are readily explained as, and probably are, dissociation with interference.*

In true reciprocal beating the impulse back from A to V probably does not retrace the path by which it has just come from V to A but goes back over fibers which were quiescent during the retrograde conduction.[14]

A-V DISSOCIATION WITH PRIMARY A-V BLOCK

Primary A-V block may properly be considered as of five degrees which may be tabulated according to the number of auricular impulses which are completely blocked and those conducted:

TABLE II

DEGREE	AURICULAR IMPULSES COMPLETELY BLOCKED	AURICULAR IMPULSES CONDUCTED
first	none	all
second	occasional	most
third	many	many
fourth	most	occasional
fifth	all	none

It is seen that the first and fifth degrees and the second and fourth degrees are counterparts.

First Degree A-V Block.—When all the auricular impulses are conducted to the ventricles, even though P-R is prolonged, there is no dissociation except if the nodal rhythm is more rapid than the auricular, that is $V > A$; in this case the result is the same as interference-dissociation, with perhaps longer P-R for the conducted beats; it will thus appear like Fig. 5.

Second Degree A-V Block.—In second degree block there may or may not be

*Of fourteen curves ten are almost certainly interference dissociation, mostly with auricular bradycardia and $A<V$; viz., White[24] gives Fig. 14 with A = 30, V = 55, and Fig. 15 with A = 43, V = 68. Dock[6] gives Figs. 3, 4, 5, 8, 9, 10; Blumgart and Gargill[5] give Fig. 1 with A = 48, V = 55, and Fig. 3 with A = 39, V = 58. Of the remaining four curves one appears doubtful but is probably actually reciprocal beating.[8] The remaining three curves[7] are without doubt reciprocal beating. There is one additional case of reciprocal beating which was missed by the author who published the curve.[3]

an occasional escaped beat after an interval p depending on three things: (1) the auricular rate A; (2) the conduction time P-R; (3) the rate V of the center just below the point of block. It should be noted that A and V depend on the two pacemakers concerned, and also on the tonus of the cardiac nerves at that time. If $pV = 60$ then the lower center escapes; if $pV < 60$ there is no escape. Such an escaped beat is usually not conducted back to the auricles, so that there is a momentary dissociation.

Fifth Degree A-V Block.—In complete A-V block the auricles respond to the S-A node and the ventricles to a pacemaker just below the point of block in the A-V node or the crus commune, with so-called idioventricular rhythm. The result is complete dissociation. Ordinarily $A > V$, but it may occur with $A \leqq V$.

CASE 3.—In Fig. 7 is shown Lead II of a case of complete A-V block. A = 78, V = 47. In addition to complete dissociation this curve shows a phenomenon of fairly common occurrence but frequently overlooked. The P-P intervals are of two kinds: those like P_1P_2 and P_2P_3 which contain an R, and those like P_3P_4 and P_8P_9 which contain no R. The nine intervals of the first kind measure 67 to 76 and average 72½; the five intervals of the second kind measure 79 to 88 and average 84. Hence the maximum, the minimum, and the average interval of the second kind are 12 each longer than the maximum, the minimum, and the average interval respectively of the first kind; and the minimum 79 of the second kind is longer than the maximum 76 of the first kind. This cannot be accidental; the same phenomenon is seen in many cases of complete and also of partial A-V block,[9, 10, 19, 22, 26] although it is often absent.

That the shortening of the P-P intervals which contain an R is not entirely dependent on vagus influence is demonstrated by its persistence, though to a lesser degree, after injection of atropin.[9] The most reasonable explanation is as follows: in ventricular bradycardia the coronary blood flow is improved by ventricular systole; the circulation of the S-A node is improved, so that the S-A node builds up and discharges its next impulse sooner.[10, 22] Some authors place the improvement in S-A conduction rather than in the S-A node.[11, 19]

Third Degree A-V Block.—In the absence of primary A-V block there can be no permanent A-V dissociation if $A > V$; but it can occur with primary A-V block. For example, let there be primary 2:1 block, A = 71, V = 40. The diagram of Fig. 2 shows the mechanism. Alternate impulses A_1, A_3, A_5, etc., are blocked in the A-V node because of the primary 2:1 block; otherwise the mechanism is the same as in Fig. 1, including the periodicity and the fixed, relatively long coupling.

CASE 4.—Let us first examine Leads I and II of Fig. 11. There are three possible explanations: (a) nodal rhythm, rate 91, with retrograde conduction; (b) sinus rhythm, rate 91, P-R = 54; (c) complete A-V dissociation, A = V = 91, with primary complete A-V block, or without any primary A-V block.

From Leads I and II of Fig. 11 we cannot determine which of the three interpretations is correct, though the deformed P of Lead I and the inverted P of Lead II favor (a). But in Lead III we see the same regular auricular rhythm with a quite irregular ventricular rhythm; this makes it quite improbable that the auricular rhythm is dependent on the nodal, and is strongly against (a) though it does not disprove it, for there might be forward block from the node but no retrograde block.

Fig. 7

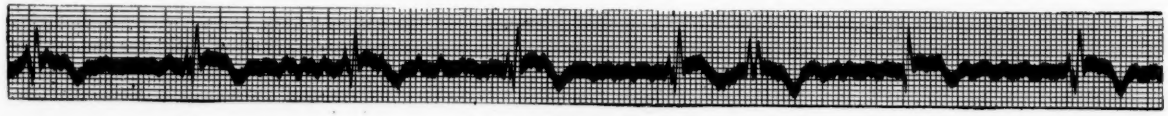

Fig. 8

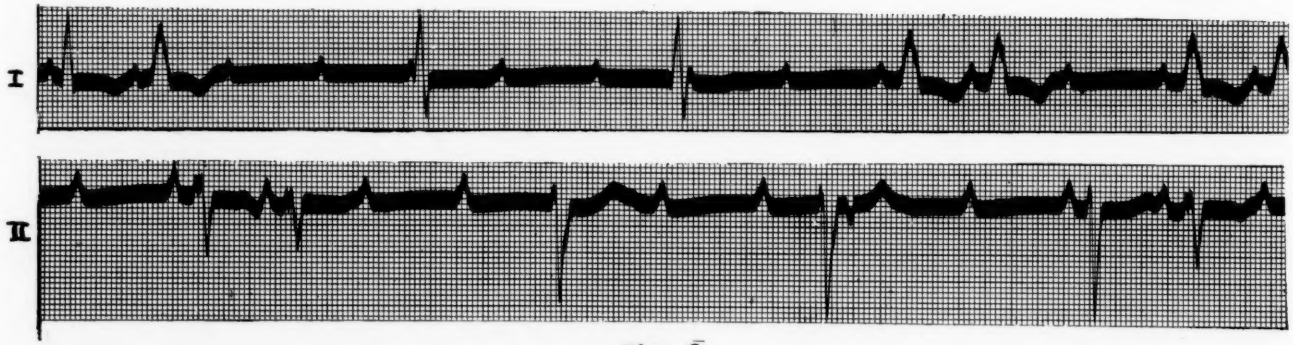

Fig. 9

Fig. 7.—Case 3. Complete A-V dissociation in complete A-V block. Variation of P-P intervals due to ventricular systole.
Fig. 8.—Case 5. Auricular fibrillation, fourth degree A-V block.
Fig. 9.—Case 6. Fourth degree A-V block, Leads I, II. Aberrant conduction. Retrograde conduction.

In Fig. 10 are shown three previous curves from the same patient taken at intervals, the top curve first. These curves clearly show sinus rhythm. There is a gradual change in the P-waves, P becoming diphasic in Lead I and negative in Leads II and III, so that the P-waves of Fig. 11 are not much different from those in the last curve of Fig. 10. This indicates more or less conclusively that the P-waves of Fig. 11 are not retrograde, so that explanation (a) is invalid. The marked irregularity of the ventricular beats in Lead III of Fig. 11 and in Fig. 12 eliminates (c), so that we are left with (b).

To explain Fig. 11* and Fig. 12 it is unnecessary to assume the presence of any escaped beats, and the marked variation of the longer R-R intervals from 112 to 125 speaks strongly against escape. The regularity of Table III also is in favor of conduction.

TABLE III

P-P	73-70	R-P	39	39	39	38	38	38	38	37	19	18	17	17	16	16	16	<14 =
P-R	32-35	P-R	52	53	53	54	54	54	55	57	–	–	65	–	62	–	–	–

In Fig. 10 the P-R of the three curves are 23, 22, and 23, and in Fig. 12, Lead I the P-R is 32 after a full rest of P-P = 70; this is evidence of some degree of primary A-V block. Fig. 12 represents sinus rhythm and third degree A-V block with no escaped beats; the block is mostly 3:2 but is occasionally 4:3 and 2:1. In Fig. 12 there is a conduction interval of 65, namely $P_9R_7 = 65$, with $R_6P_9 = 17$. The absence of dropped beats in Leads I and II of Fig. 11 is due to a lesser degree of block at that time, as shown by the shorter P-R in Fig. 11, Lead I in spite of shorter R-P.

In passing I merely call attention to an instance of sinoauricular block in Lead III of Fig. 11, in which P_6 is dropped.

Fourth Degree A-V Block.—The degree of A-V block may be great enough so that for long intervals no beats are conducted, and only occasionally is there conduction.

CASE 5.—In Fig. 8 is shown a case of fourth degree block. The auricles are fibrillating, at a rate of about 460; almost all the auricular impulses are blocked, and the ventricles respond to a pacemaker in the crus commune with a rate of 50. Only one auricular impulse is conducted.

CASE 6.—(A male of seventy-four years.) For forty-four years this man had had a pulse rate of about 30, and for the past three years he had Stokes-Adams attacks. In Fig. 9 it is seen that only five P-waves are conducted to the ventricles in Lead I, and only three in Lead II; there is, therefore, a high degree of primary A-V block. The abnormality of the conducted ventricular complexes is doubtless due to aberrant conduction in the bundle. A-V conduction, when it occurs, is due to a temporary improvement (circulatory or otherwise) in the diseased portion of the bundle, and the conduction of a second beat is probably partly due to the local circulatory improvement caused by the ventricular systole preceding. This view is favored by the history of Stokes-Adams attacks, which are due to temporary relapses in the diseased portion of the bundle.

*To conserve space only part of strips I and II (for which Table III is made) are reproduced.

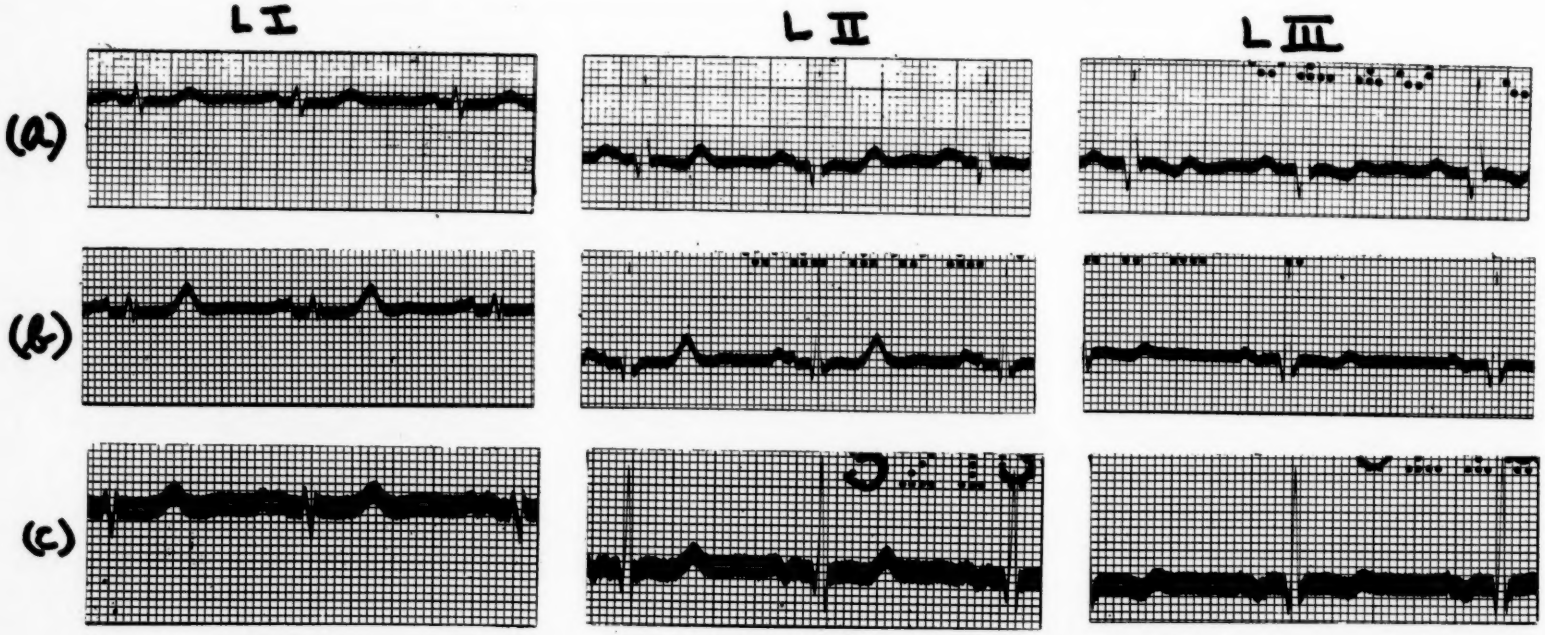

Fig. 10

Fig. 10.—Case 4. Three curves at intervals of several months, the top curve first; Leads I, II, III from left to right. First degree A-V block. Note gradual change in P-waves.

An interesting phenomenon is the retrograde conduction seen in Lead II of Fig. 9. This was frequently repeated in unpublished parts of the curve. It seems that retrograde conduction is not at all uncommon in high degrees of A-V block.[27]

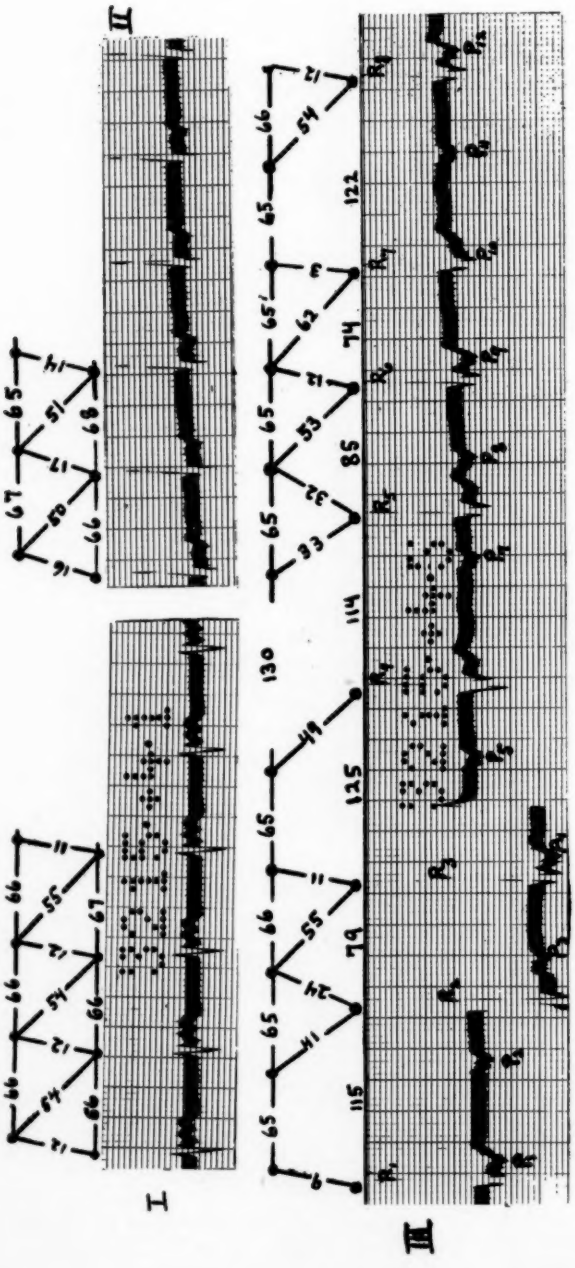

Fig. 11.

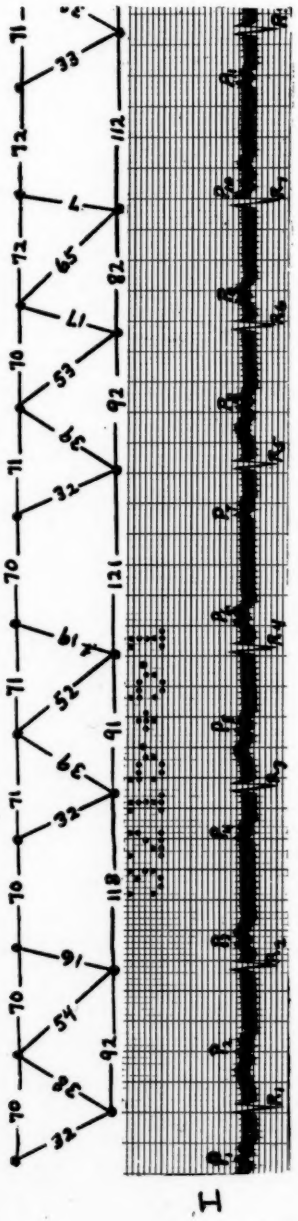

Fig. 12.

Fig. 11.—Case 4. Sinus rhythm. First and second degree A-V block. No escaped beats. Sinoauricular block at P_9 in Lead III.

Fig. 12.—Case 4. Sinus rhythm. Third degree A-V block (4:3 and 3:2), Lead I. Conduction interval P_6R_7 of 0.65 second.

TOPICS RELATED TO DISCUSSION OF A-V DISSOCIATION

The Supernormal Phase.—There are a number of recorded cases in the literature of third or fourth degree A-V block in which only auricular impulses with short recovery periods are conducted, while those with longer recovery periods

are blocked. This apparently paradoxical occurrence is explained by Lewis and Master[13] by assuming a supernormal phase, according to which the passage of an impulse through the A-V node is followed by a short period of supernormal conductivity, so that an auricular impulse very early in diastole is conducted, whereas one falling later in diastole falls outside the supernormal phase and so is not conducted. Ashman and Herrmann[1] also give curves showing this phenomenon.

It is necessary first of all to state precisely what is meant by a supernormal phase. In the discussion of complete A-V block it was mentioned that in ventricular bradycardia the blood circulation of the S-A node is improved by ventricular systole; the same is probably true of the A-V node and bundle; this might very well increase A-V conductivity. Changes in tonus of the cardiac nerves may also increase A-V conductivity. Hence an apparent supernormal phase may be due to improved coronary blood-flow or to changes in tonus of the cardiac nerves such as perhaps occurs in Figs. 8 and 9; but this cannot properly be called a supernormal phase, a term which should be applied only to a phase of increased conductivity due solely to the passage of a previous impulse over the tissue in question, as in the case of an isolated nerve-fiber. In neither of their cases have Lewis and Master excluded changes in tonus of the cardiac nerves, and in their second case they may be dealing with ventricular extrasystoles. The occurrence of a true supernormal phase of A-V conductivity has not been demonstrated.

Very Long P-R Intervals.—The question has been frequently raised as to how long the P-R interval may be. Barker and Bridgman[2] state that there is no upper limit to the possible length of P-R; this statement is unquestionably wrong, for there can be no doubt that if an auricular impulse is not conducted within say an hour it will not be conducted at all, so that P-R can never be greater than one hour. The important question is, however, not how long we think P-R may be but how long it actually is; in other words, what is the longest P-R ever recorded? Before we can speak of a P-R interval we must be sure that the R is actually due to conduction of the impulse from P, and this is frequently not easy to decide.

There are many recorded P-R intervals of 40 to 50 (in hundredths of a second) though they are uncommon. In Case 4, Fig. 12, we have a P-R interval of 65. The only longer unquestionable P-R conduction intervals I have seen are in cases of Thayer[20] and Herrmann and Ashman,[12] in which there are P-R intervals as long as 73. But several authors have reported longer P-R intervals, ranging from 80 to 103.*

Fig. 11.—Case 4. Sinus rhythm. First and second degree A-V block (4:3 and 3:2), Lead I. Conduction interval P_8R_7 of P_8 in Lead III.

Fig. 12.—Case 4. Sinus rhythm. Third degree A-V block (4:3 and 3:2), Lead I. Conduction interval P_8R_7 of 0.65 second.

*I should like to discuss some of these cases in a little detail. Barker and Bridgman[2] believe they have P-R intervals of 90 to 103. Careful analysis of their curve demonstrates that the longest actual P-R interval is 43; the R which they say are conducted after the intervals 90 and 103 are clearly escaped beats following block of the preceding auricular impulses. Thayer[20] reports a case with P-R interval of 102. In his electrocardiograms the P-R intervals vary from 64 to 73; on the same day on a polygram is marked an a-c interval of 102, followed two beats later by an a-c of 60; the long a-c interval is quite unconvincing, so that the longest conduction time is 73. Wenckebach and Winterberg[23] show a curve in which they believe there is a P-R interval of 96. A diagram was made showing their interpretation; the P-waves are all alike and the R-waves are all alike. There is obviously more than moderate A-V block present, inasmuch as there are twenty-two P-waves and only twelve R-waves. With such a degree of A-V block it is unlikely that there should be as rapid conduction as they indicate: P-R = 11 to 14 in 7 places, all after recovery periods of from 48 to 54, whereas in normal sinus rhythm in the normal heart the recovery period[26] is 65 and only uncommonly shows a conduction time of as short as 11. A priori it seems unlikely also that a P-wave should be conducted to an R with an intervening R, so-called skip-conduction, but this objection alone is not valid, as we shall see. There are two other possible explanations of their curve, both I think more likely than theirs. First, we may be dealing with A-V dissociation, complete except for interference, with auricular tachycardia of 183 and nodal tachycardia of 94. Secondly, there may be 2:1 A-V block (except at one place) with a maximum P-R of 40. Ashman and Herrmann[1] believe they have a P-R of 101; there seems to be no conclusive reason for assuming skip-conduction in their curve, so that we cannot accept their long P-R. It would be equally justifiable to assume skip-conduction in our Case 6, which would yield us a P-R of 88, namely $P_{10}R_9$ in Lead I of Fig. 9.

Skip-Conduction.—By skip-conduction I mean conduction from an auricular impulse P to a ventricular impulse R with an intervening ventricular complex R_1, as shown in Fig. 3. Can this occur, and if so, how? Clearly R_1 cannot originate in the A-V node, with a normal conduction time, for otherwise P would be blocked and could never reach the ventricles to cause R; hence R_1 must originate below a region of block, viz., at a point O in the crus commune (Fig. 3); this presupposes a region of block B just above O. It is also clear that R_1 must be discharged from O before P reaches O, and furthermore, that R_1 is not conducted upwards, for otherwise it would meet and block the downcoming P. Since P is to be conducted through O it must reach O a considerable time after O discharges R_1, which can occur only because P is delayed long enough in B. If then we assume such a region of block B with partial forward and complete retrograde block it is theoretically possible to have skip-conduction as shown in the various segments of Fig. 12. That this actually occurs is demonstrated conclusively by Scherf's[18] case in which there is skip-conduction of type (a).

Crus Commune Block.—With a region of block in the crus commune as discussed above it is clear that the minimum coupling interval R_1R (Fig. 3) varies as the absolutely refractory period of the center O plus the conduction time from O to the ventricles, which is shorter than the conduction time from the A-V node to the ventricles. In this way can be explained cases of A-V dissociation with interference in which the interval of coupling is relatively short, and shorter than that in interference-dissociation. The mechanism is similar to that of interference-dissociation, in analogy to Fig. 1 and Fig. 2.

Organic Block.—Before we can conclude that A-V block is organic we must first show that it is primary; then we must be sure that it is not chemical (e. g. digitalis); and finally we must exclude vagus influence, which is best done by injection of atropin.

SUMMARY

1. A-V dissociation is the condition in which the auricles and the ventricles are controlled by different pacemakers; if occasionally they respond to the same pacemaker the condition is incomplete dissociation or dissociation with interference; otherwise it is complete dissociation. Complete A-V dissociation and complete A-V block are by no means the same thing.

2. Primary or essential A-V block is block due to prolongation of the refractory period of the A-V node or the bundle; adventitious A-V block is block due not to prolongation of the refractory period but to the fact that the impulse reaches the A-V node or the bundle during its normal cyclic refractory period.

3. A-V dissociation of any degree, even complete, may be present without any primary A-V block; this is interference-dissociation, and occurs only if $A \leqq V$.

4. In a regular series of supraventricular complexes interrupted by shorter R-R intervals dissociation with interference must be considered, especially during the administration of digitalis.

5. Reciprocal beating of the heart is the condition in which an A-V nodal impulse is conducted back to the auricles, and then this auricular impulse is conducted forward again to the ventricles. Most of the curves reported as reciprocal beating are really interference-dissociation. There are actually four or five reported cases of true reciprocal beating.

6. Primary A-V block is divided into five degrees instead of the four degrees heretofore considered.

7. In many cases of complete and of partial A-V block, the P-P intervals which contain an R are shorter than those which do not. This may be due to improvement of the circulation to the S-A node by ventricular systole.

8. A true supernormal phase of A-V conduction denotes a phase of increased conductivity due solely to the passage of a previous impulse through the A-V node; this has never been conclusively demonstrated to occur.

9. Those P-R intervals recorded of from 0.80 second to 1.03 seconds probably do not represent conduction but escaped beats. The longest recorded P-R conduction interval which can be accepted without question is 0.73 second. A case is shown with a P-R conduction interval of 0.65 second.

10. Skip-conduction is conduction from an auricular impulse to a ventricular impulse with an intervening ventricular complex. Theoretically this is possible with a region of partial forward and complete retrograde block in the crus commune. A case is referred to in which skip-conduction actually occurs.

11. Before concluding that A-V block is organic it must be shown that it is primary and not adventitious, that it is not due to digitalis or anoxemia, and finally that it is not due to vagus influence which is best tested by atropin.

12. The foregoing discussion permits a classification of A-V dissociation into the following categories (aside from the obvious instances in early auricular extrasystole, ventricular extrasystole, and paroxysmal ventricular tachycardia):

Without primary A-V block

$A < V$ (Auricular bradycardia, nodal tachycardia)—interference-dissociation

rarely $A = V$—complete dissociation

With primary A-V block

2nd degree A-V block—escaped beats

3rd and 4th degree A-V block { $A \neq V$—dissociation with interference; $A = V$—complete dissociation }

5th degree A-V block—complete dissociation

I wish to express my indebtedness to Dr. Louis N. Katz, at whose instigation this research was undertaken, for his stimulating discussion and criticism.

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THE CONTROL OF POLLEN ALLERGY*

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WHILE sensitization to pollens may be responsible for attacks of pollen dermatitis or eczema or even gastrointestinal manifestations, by far the commonest symptoms of pollen allergy are hay fever and asthma. The doctor in the past has given little attention to the treatment of hay fever and asthma due to pollens, because he has found that not much can be done for these sufferers. Even the treatment with pollen extracts, first hailed as a great panacea, was found to fail so often that in many sections it became quite discredited. Indeed, even today it is true that pollen therapy as administered by the majority of physicians is more often disappointing than otherwise.

Incidence.—When properly applied, pollen therapy will give practically complete relief to two-thirds of seasonal asthmatic and hay fever cases. When properly applied fully 90 per cent will obtain adequate relief, sufficient for them to be entirely satisfied. If these two statements can be substantiated, and I shall have no difficulty in doing so, we are justified in giving some thought to the problem of hay fever and asthma due to pollens. There being between 3 and 4 million such cases in this country, the problem becomes one that touches every man interested in the general practice of medicine. Balyeat gives a rough estimate of 2 per cent of the population suffering from hay fever or asthma. Piness and Miller found, in a careful survey of two populations totaling 4,000 people in the West, that 4.4 per cent of one and 3 per cent of the other were hay fever victims. Cooke and Vander Veer in a similar survey in New York found 3.5 per cent of the population suffering from asthma or hay fever. If *one out of thirty* in the population at large suffers from hay fever or asthma from one cause or another, allergic therapy must necessarily be of interest to the average physician. Indeed, it has been estimated that *one of every fourteen,* suffers from some form of allergy, and this estimate is decidedly conservative.

The question before us is, what is adequate pollen treatment and how should it be given; by what measures may we obtain the excellent therapeutic results just mentioned?

Inhalant allergy, asthma, hay fever, and vasomotor rhinitis are due to a variety of causes; pollens, house dust, feathers, animal epithelia, orris root, occupational dusts, pyrethrum and other less frequent causes. The appropriate

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treatment necessarily varies depending upon the cause. The chief among these however is the pollens.

The first thing to remember is that pollen allergy is seasonal. It does not persist throughout the year. As usual there are exceptions, such as workers in greenhouses and those who live where pollen is in the air throughout the year, as in Texas and Southern California. But the general rule holds. Always suspect pollens when the symptoms are worse during the warm months of the year.

DIAGNOSTIC REQUISITES

What are the requirements for adequate diagnosis of the cause of pollen allergy?

A Botanical Survey.—First one must know what hay fever plants are indigenous to the territory in which the patient lives and the period of their bloom. This was one of the major problems confronting allergists early in their work. Excellent botanical treatises were available, detailing the distribution of the flora of the country, but they were too general for reliable use in this work. The early tables of distribution, prepared by the manufacturers of pollen extracts were based chiefly upon these general surveys and were in no sense accurate for specific localities. It became necessary to make local botanic surveys throughout the country, paying especial attention to those trees, grasses, and weeds which have in the course of years been found responsible for allergic manifestations. Fortunately, such studies have been made, over sufficiently representative sections of the country and are available for reference so that, with their aid, the man not primarily interested in allergy can develop a fairly satisfactory knowledge of the more important hay fever producing plants in his vicinity. Obviously it is illogical to administer ragweed extract on the Pacific Coast where ragweed is not a factor, or Russian thistle, a frequent offender of the West, in Virginia where it is of little importance, even though the patient has reacted positively thereto.

A Pollen Calendar.—One must be well acquainted with his pollen calendar. A young lady with hay fever always beginning in August and extending to frost had been treated for three successive years with ragweed extract, never with benefit. Had the doctor known that ragweed does not start to pollinate in Virginia by the tenth of August, the date on which this patient's symptoms usually begin, he would have searched elsewhere for the cause. This fact being known to us, we promptly found the cause in goldenrod and were able to give the patient adequate relief.

For best results one must also be somewhat of a field botanist. It is not enough to know what is pollenating but also how abundantly. The last pollen season in Virginia, has been the heaviest on record. During this season I took frequent rides into the country to see what was pollinating and how abundantly. While ragweed was everywhere I observed that goldenrod was almost as abundant. Acres upon acres were almost uniformly yellow with this pretty flower. Now, goldenrod is usually said not to be much of a factor, since the pollen is heavy and sticky and the plants are insect pollenated. Goldenrod pollen is said not to carry more than a few hundred yards. Sensitization to goldenrod is not at all uncommon in my experience, but we usually do not desensitize against

it because the patient can so easily avoid it. But this year with the roadside lined for mile after mile with this plant, nearly any drive into the country would result in adequate exposure to produce symptoms. Furthermore we have found goldenrod pollen on our slides on the eighth floor of an office building in the center of Richmond. A child entirely satisfactorily desensitized against ragweed to which she was sensitive did have some symptoms this last season which were unexplained, until I visited her home on the outskirts of town and found that she was playing most of the day in a vacant lot next door, quite overgrown with goldenrod. Her teacher also had goldenrod in the schoolroom. Coseasonal treatment was then instituted with goldenrod extract to which she was sensitive, with prompt subsidence of symptoms. It is well therefore to bear in mind that if your patient is not satisfactorily relieved, the cause for failure may be other than inadequate desensitization with the pollen or pollens with which you have been treating him.

While a very great variety of plants may be responsible for allergic symptoms, less than two dozen are responsible for fully 90 per cent of the trouble. The problem of acquiring practical experience in field botany is therefore not a very difficult one.

As a corollary to the above, we need but mention that the pollen extract used for diagnostic testing must be appropriate. One must use extracts of those hay fever producing pollens to which one knows that the particular patient will be exposed. For the man who wishes to get good results in a reasonable percentage, say 75 per cent of his patients, and who plans on sending the more difficult ones to an allergist who has a greater variety of pollens at his disposal the necessary list of extracts is not large. The list however varies in different sections of the country.

Potent Test Extracts.—The extracts must be potent. Most of those available today through the pharmaceutical concerns are potent. With different methods of extraction the duration of the potency varies and one should therefore be acquainted with the durability of his extracts.

Reliable Test Methods.—The methods of testing must be reliable. Both the scratch technic and the intradermal method have their advocates. Provided one has a potent extract such as a two per cent extract, the scratch method will be found reliable in about 90 per cent of cases. This is true of pollens but not equally so of several of the other allergens. The factor of safety is so much greater with the cutaneous or scratch method, when we are dealing with pollens that it is the only method which can justifiably be recommended, in my opinion, to those who have not made a special study of allergy. Here again I am speaking only of the pollens.

I should mention a few points which in my experience have increased the accuracy and safety of diagnostic pollen testing.

Always perform pollen tests on the arm. If this is done the very rare untoward systemic anaphylactic reaction can be promptly controlled by the application of a tourniquet above the site of the reaction and the injection of 0.3 c.c. of adrenalin into the opposite arm.

Place the scratches at least one inch, preferably an inch and a half apart.

Too often the scratches are placed so closely together as to result in fusion of positive reactions and confusion in reading.

If the date of onset of symptoms makes you reasonably certain that a certain pollen or group of pollens is responsible, and if the skin reactions have been negative, repeat the test. False negative reactions are a distinct source of error. I have seen a frank negative in one test become a strongly positive reaction on another test made half an hour later.

If in spite of continued negative reactions you still have a reasonable doubt, the ophthalmo reaction may be applied. This method has been recommended especially by Peshkin and I have found it of distinct value. A small amount of natural dried pollen on the tip of a flat toothpick is introduced inside the lower lid. Five minutes later this is washed out with physiologic saline. The intensity of a positive reaction varies from a little redness at the inner canthus to an intense congestion of the conjunctival mucosa with some edema. The last is rare unless the skin test has been positive, and the test should not be used in the event of a positive skin reaction. The reading is made by comparison with the untreated eye, after which the reaction is promptly controlled with a drop or two of solution containing one part of adrenalin 1/1,000 and three parts of boric acid solution.

Do not desensitize with everything that gives a positive reaction. Use only those pollens which coincide on the pollen calendar with the dates of symptoms. As a rule, use only those that are wind pollenated. In my experience it is almost a rule to observe positive reactions to pollens whose presence have not caused symptoms, in addition to those which have.

THERAPEUTIC REQUISITES

I have discussed briefly some of the more important factors in the diagnostic study. Naturally those who will be making these studies at all frequently will refer to some of the excellent recent volumes on allergy which are now available. Many prefer to send their patients to an allergist for diagnostic survey, and these are more particularly interested in the most appropriate method of treatment after the patient's reactions have been determined and he has returned for treatment by his home physician.

Potent Extracts and Adequate Dosage.—Among the requirements for successful treatment we must again mention the use of potent extracts. Next comes adequate dosage. A patient half desensitized is almost better not desensitized at all. I can usually obtain better results during the pollen season with a patient who comes in without having had any previous treatment than with a patient who has had half a course of treatment and then comes in with symptoms. One of the most pernicious hindrances to good results today in pollen therapy is the commercial fifteen dose treatment set. At the completion of the fifteen doses the patient is usually only about half desensitized, but since he took the whole set, the patient and usually his doctor also, settle back with the assumption that adequate treatment has been given. Then, when symptoms return at the usual date, both patient and doctor conclude that there is nothing to the treatment. These sets usually provide a top dosage of three thousand units. In Virginia, where

the ragweed concentration is relatively low, I always try to provide a maximum dose before the season of twenty thousand units. This is one of the most important requisites of preseasonal treatment. Some allergists in sections of the country where the ragweed count is high, provide even higher dosage.

It is unfortunate that discussion of dosage today is complicated by the rather general use of three entirely different terminologies, all of them quite arbitrary. The pollen unit is more frequently used by the commercial houses. Its advantage lies in the avoidance of the use of fractions. One pollen unit represents the active principle extracted from one millionth of a gram of dried pollen.

A second nomenclature is based upon the strength of the pollen extract in terms of nitrogen content. If all of the proteins in pollen were antigenic and if the antigen contained nothing other than protein, dosage based upon nitrogen content would be entirely satisfactory. Unfortunately, however, it has been demonstrated that the antigenic activity of a pollen extract does not parallel its nitrogen content. This appears to be due to the fact that a given pollen contains several proteins not all of which are antigenic and that other substances besides proteins, particularly polysaccharids enter into the antigenic reaction.

The third system for recording dosage is the simplest. It is probably the more frequently used by allergists. It consists merely in recording the strength of the extract as prepared. Thus, a concentrated 2 per cent extract is called 1/50. This, diluted ten times or a hundred times is designated 1/500 or 1/5,000.

Since, in your reading, you will come across the first and third classifications the more frequently, it will suffice to say that 1 c.c. of 1/50 concentration contains 20,000 pollen units, 0.1 c.c. of 1/50 contains 2,000 pollen units. The fifteen dose treatment set usually starts with a first dose of something under 20 units and ends with a top dose of 3,000 units. In the average, that is, not the extremely sensitive case, the allergist usually starts with 0.1 c.c. of 1/5,000 and increases the dose steadily to a top dose around 1 c.c. of 1/50. This top dose is obviously over six times as strong as that provided in the treatment set and the results are usually proportionately more effective.

Control Tests.—A third desideratum for successful therapy is the development of some method of testing during the course of treatment by which we may determine when the patient has been adequately desensitized. The cutaneous reaction is not entirely satisfactory as a control test, since one may have hay fever even though the cutaneous sensitivity has been entirely abolished and, vice versa, one may be entirely relieved of hay fever by treatment even though the skin test is still positive. However, this serves as a fairly satisfactory rough method of check and one should always attempt to give sufficient treatment to render the patient negative to the cutaneous scratch reaction with a concentrated (1/50) extract.

Realizing that there is this difference between cutaneous and mucous membrane sensitivities, and that our major interest is in rendering the patient's mucous membranes insensitive to ragweed, I have attempted during the last ragweed season to develop an eye test comparable to the diagnostic ophthalmo reaction previously mentioned, and which will give us more definite information prior to the onset of the season regarding the sensitivity of the patient's mucous

membranes. For this test we use saline extracts of the dried pollens in concentrations of 5 per cent, 0.5 per cent and 0.05 per cent. These we designate as 1/20, 1/200 and 1/2,000. The test is applied by introducing a drop of saline extract onto the ocular conjunctiva and studying the intensity of the eye reaction. The direct application of the dry pollen as in the ophthalmo test is not satisfactory for this purpose since it provides a concentration in the eye far in excess of any that will be met with during the pollen season. While we have not completed the study of our results, I will say that three-fourths of the patients whose ocular conjunctivae are negative to 1/20 concentration will get through the season with more than 90 per cent freedom from symptoms. Of those who are sensitive to 1/20 but insensitive to 1/200 two-thirds will have more than 90 per cent relief. These tests were applied two or three weeks before the onset of the season, and with those who appeared from the test not to be adequately desensitized, treatment was pushed more intensively. Their tests showed satisfactory improvement just prior to the onset of the season. Had we not been applying this preliminary test, we would have failed to give especially intensive

TABLE I
THERAPEUTIC OPHTHALMO REACTION

100% RELIEF WAS OBTAINED IN:	
50% of cases who were	negative to 1/20
33%	negative to 1/200
8%	postive to 1/200
90% AND 100% RELIEF IN:	
71.4%	negative to 1/20
66.6%	negative to 1/200
41.6%	postive to 1/200

treatment to those who apparently require more than the average, and they would not have been as satisfactorily relieved. I believe that as soon as we have completed the standardization of this therapeutic eye reaction we will have available a much more valuable and direct method for controlling the progress of treatment.

Direct Control.—The doctor who has direct supervision of his patients will obtain better results on the average than the man whose patients are being treated through the intermediary of another physician. This is especially true of coseasonal treatment. We have developed a fairly standard method of preseasonal desensitization, but once the season has begun, the treatment varies from day to day depending upon the pollen concentration of the air. This last ragweed season was unusually heavy and the coseasonal treatment that I had found satisfactory in 1930 was found to be inadequate in 1931. The much higher pollen concentration in the air required a compensatory reduction of the size of coseasonal dosage, to maintain the balance. In Richmond, where we knew the amount of pollen in the air each day, we could grade our coseasonal dosage accordingly. But with the patients out of town, the time required in correspondence to and from the doctor prevented this ready flexibility and the results with out of town patients were sometimes not quite as good as with those who reported to the office.

Daily Pollen Graphs.—The last of the important requisites for successful therapy is a *sine qua non.* These are the pollen graphs. No man should seriously attempt to carry on pollen therapy without knowing from day to day the pollen prevalence in the air. The procedure is very simple. An ordinary microscope slide coated on one side with white vaseline is exposed on a window ledge or on the roof, slightly protected from rain, and in the shade. Every twenty-four hours the plate is taken in and a new one replaced. The pollens are actually counted under the microscope. A mechanical stage is helpful. For standardization, the barrel of the microscope is drawn out until the diameter of the field is exactly 1.4 mm. This can easily be done by focusing on the squares of a hemocytometer chamber. For ragweed, a count of five successive trips across the narrower width of the slide expresses the number of pollen grains per cubic yard of air per twenty-four hours. If we wish to always express our figures per cubic yard of air, the area of the field counted will vary, depending upon the size of the pollen grains, but for practical purposes this is not necessary, since the variation is not great and our interest is usually in the relative counts of the same pollen from day to day.

With the information obtained in this way, we can plot the curve of pollen concentration for each day. In my own office this is recorded each day on a blackboard so that the patient also may study the curve (Fig. 1). If, on days in which the pollen concentration has been unusually high, he has some symptoms he can understand the reason therefor and know that it was due to a temporary unusual prevalence and not to failure of treatment. Such a patient might have two or three days of hay fever or asthma during the season but he understands why, and is not dissatisfied. When asked a year later what results he obtained he will say, "I did splendidly except on two or three days, when the pollen concentration was unusually high and when I had some symptoms." A patient who has not studied such a chart and who expects to get complete relief will only remember that he did have symptoms after all. A year later he will say, "I had the treatment but I had asthma just the same." But the chief value of a pollen graph is in its control of coseasonal treatment, since as I have said, on those days on which the pollen prevalence is high, the coseasonal dose must be proportionately reduced.

Some years one will feel that he is obtaining consistently good results from his pollen therapy, and the next year it may be that with the same treatment the results are disappointing. The pollen graph will show however that on different years the pollen concentration varies decidedly (Figs. 2 and 3). Variation in results was due not to the treatment but to the pollen concentration. Meteorologic conditions are chiefly responsible for the varying pollen concentration. In 1930 Virginia was hard hit by the drought. There was no rain in August until the twenty-eighth when we had a heavy rainfall of 1.8 inches. There was as a consequence a ten day delay in the onset of the pollen season and hay fever victims who had had no treatment were jubilant with the thought that at last they were free of the disease. But there is an old saying, "nothing is certain in the future save death, taxes and hay fever." Within three days after the rain of August 28, ragweed commenced to bloom and, making up for lost

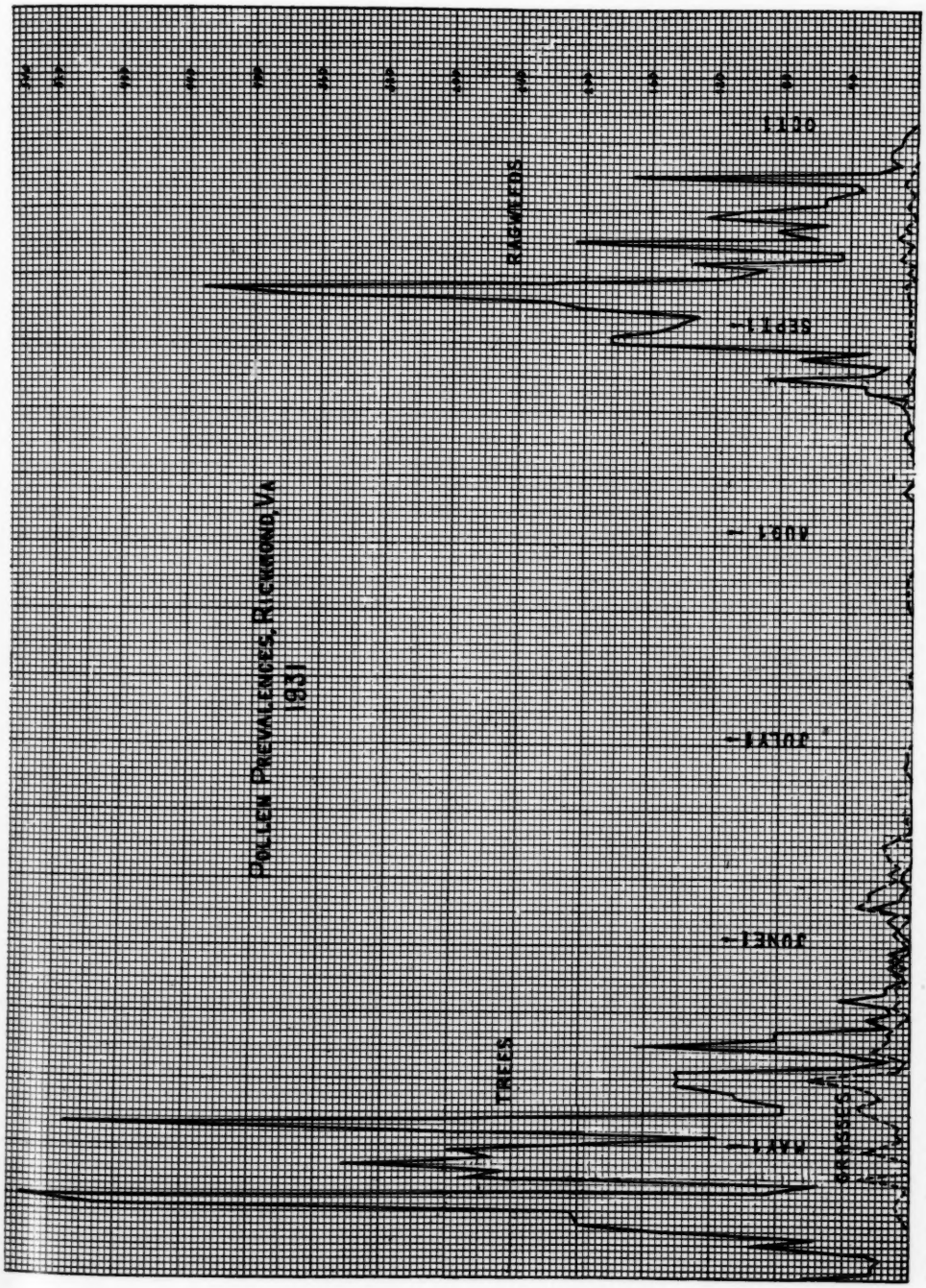

Fig. 1.—Pollen prevalences in Richmond in 1931. The tree curve represents all varieties of tree pollens. The ragweed curve therefore represents the heaviest individual pollen prevalence for year.

time, shed in unusual abundance, so that after the first of September we had a pollen concentration heavier than the average (Fig. 2).

The 1931 curves are most interesting (Fig. 3). We had drawn a probable curve before the onset of the season representing the mean of the preceding four

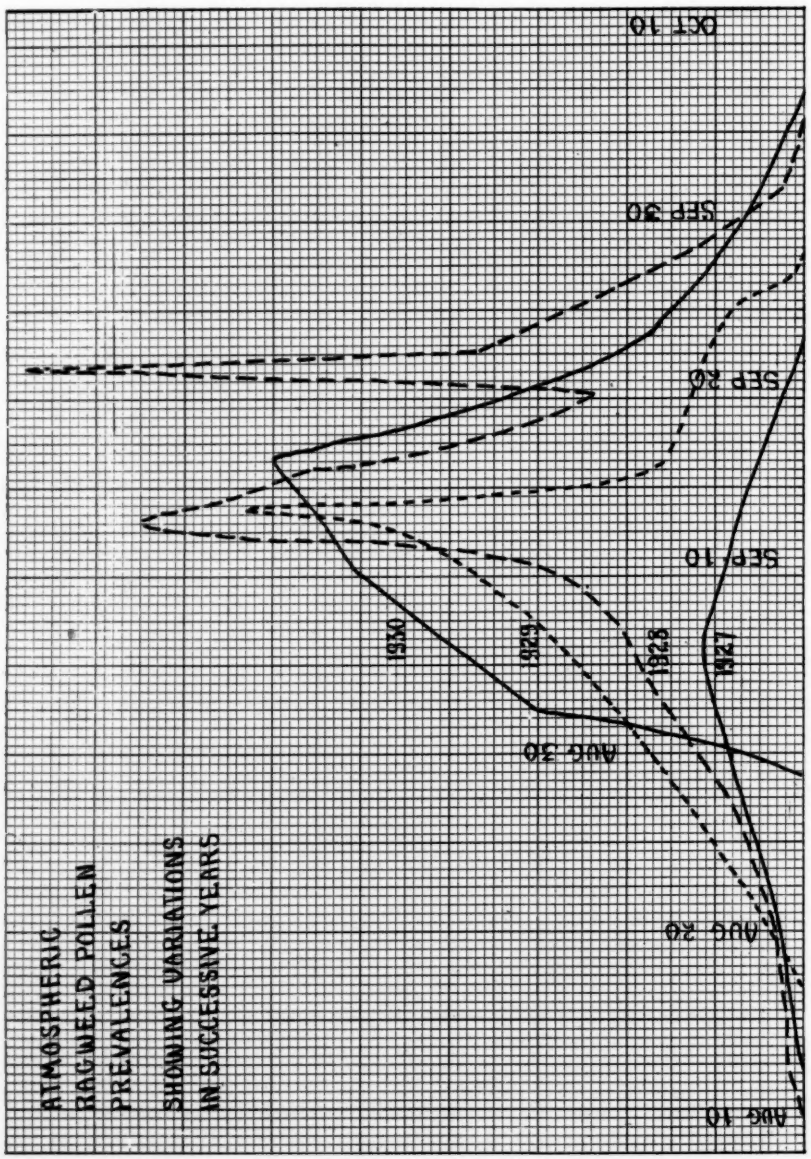

Fig. 2.—Variations from year to year. Mean curves. The delay in the start of the 1930 curve was due to the drought of that year. After pollen distribution once started it continued, heavy average.

years. To our surprise ragweed first appeared on pollen plates a week earlier than usual, and the concentration through the season ranged from three to seven times heavier than any other season since we first began keeping records. This unusually high prevalence of 1931 appears to have been rather nation-wide. As far as Virginia is concerned there appear to have been three causes. First, as we have just seen, in 1930, when it finally started there was an unusually heavy

distribution of pollen, and therefore an unusually heavy fertilization of seed. Second, July in Virginia was unusually hot, the average high temperature being 80, two degrees hotter than usual. But the rainfall was normal, 4.5 inches. Therefore, ragweed like all other vegetation grew luxuriously in July. Third,

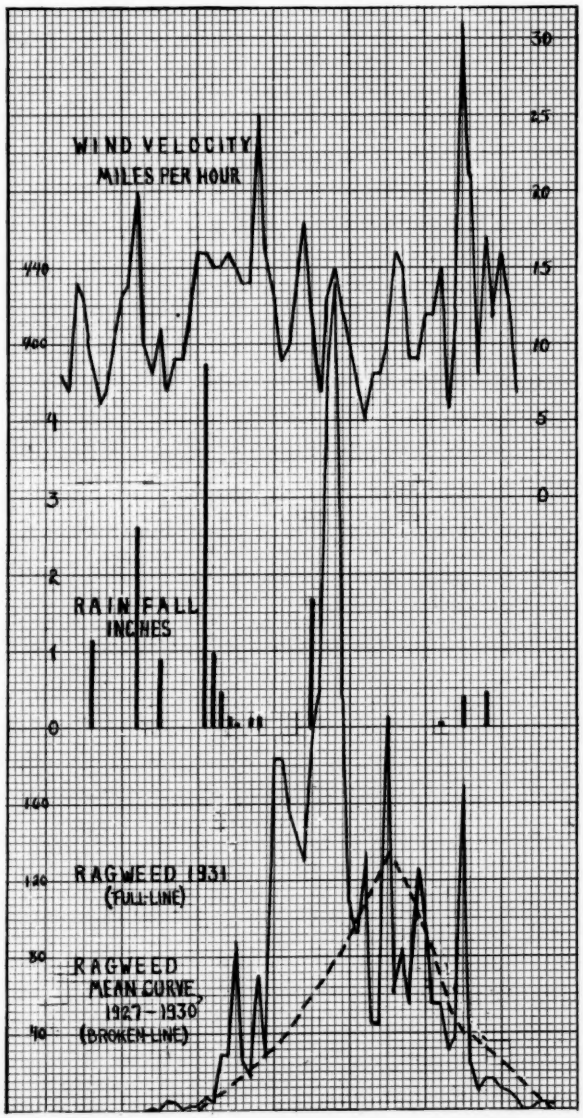

Fig. 3.—Showing the effect of high winds in producing an unusually high pollen prevalence in the first half of the 1931 season. Also towards the end of the season. Dotted line represents the mean curve for the preceeding four years.

in August we had the heaviest rainfall (11.4 inches) that has been recorded since the weather bureau was started in 1872. These three factors, wide distribution of seed, abundant heat and sunlight, and plenty of water combined to cause the unusual prevalence of this year. Add to this several days of heavy wind early in September, which stirred large amounts of pollen into the air. Very early in

this season we discovered that the coseasonal maintenance dose of pollen extract which worked beautifully last year was causing reactions. The answer was at once apparent in the higher atmospheric concentration and we promptly cut the size of our coseasonal dose. Without the use of our pollen graph we would not have cut the dose, patients would have had frequent reactions, and the results this year would have been poor. So, it is obvious that pollen graphs are essential for best results.

The graph for one locality may not be usable in another. The wind that precedes a storm always stirs large quantities of pollen into the air and local showers may therefore cause curves to vary in different localities. Ragweed pollinates at different times on the mountain and in the valley a mile or so away. The curves also vary for different sections of the country. Durham has shown that pollination begins and ends earlier in the north and in the central states than in the south. The average concentration likewise varies. The average prevalence along the Atlantic Coast is always lower than that of the Mississippi Basin.

With potent therapeutic extracts, with knowledge of the requisite top dose for preseasonal treatment, with appropriate tests for control of the rapidity and thoroughness of desensitization and with knowledge of the pollen concentration of the air during the season, one is properly equipped for therapy.

THERAPEUTIC METHODS

There are in general four methods of treatment, preseasonal, coseasonal, perennial and rush.

Rush Inoculation which is being cautiously studied by Freeman in London has not and probably will not come into general use in this country. It consists in hospitalizing the patient and giving inoculations at intervals of hours instead of days, building up to top dose within a matter of a few days. The possibility of anaphylactic reaction following the giving of such tremendous doses in a short period is great and the patient must therefore be hospitalized and under closest supervision to control reactions when they do arise. In England, where pollenosis is due to the less toxic grasses, this may be possible but in this country where the chief offender is the much more toxic ragweed, it is a method of treatment that must be approached with the utmost caution.

Preseasonal Treatment, with injections once or twice weekly has been the common method of desensitization. When properly given this will furnish adequate relief in fully 75 per cent of patients. The most important factor in proper administration is the reaching of an adequate maximum dose prior to the onset of the season. In some localities 0.2 c.c. of 1/50 concentration or 4,000 pollen units will suffice. A safer minimum top dose is 0.5 c.c. or 10,000 pollen units. One cubic centimeter of 1/50 or 20,000 pollen units will protect in almost any locality in the United States. With some patients this high dosage must be approached with caution. The extremely sensitive may experience anaphylactic reaction when approaching this top dose. In this case treatment must be increased more slowly. I have had but one patient who has not eventually tolerated a dose of 20,000 units without trouble. And if this dose can be reached prior to the season, I feel confident of satisfactory if not complete relief irrespective of whether or not control tests have been done.

One occasionally finds an extremely sensitive patient who cannot safely receive the initial dose of 0.05 c.c. of 1/5,000 or 10 pollen units. The most sensitive that I have encountered had to receive an initial dose of 0.1 c.c. of 1/2,000,000. Therefore, in order to safeguard against this occasional extremely sensitive individual it is best before starting treatment to do scratch skin tests with the dilutions of pollen extract, 1/50, 1/500 and 1/5,000 and 1/50,000, and to start treatment with that concentration which just fails to give a positive skin reaction.

There is a rapid preseasonal method applicable to those who present themselves for treatment too late for one to attain the top dose by gradual increases at weekly or semi-weekly intervals. Suppose a patient reports for desensitization just two weeks prior to the onset of the season. Here, the treatment may be given daily or even twice daily but the doctor must be more alert for anaphylactic reactions and must be prepared to control them.

Coseasonal Treatment.—Originally it was thought that desensitization treatment could be given safely only before the season and that, once the season had commenced, treatment must be discontinued. Otherwise there was great danger of anaphylactic reaction. For the patient to whom preseasonal treatment did not give adequate relief there was nothing more to be done.

In 1923 the speaker first described the treatment of a series of patients who presented themselves for the first time during the season and without previous therapy. Since then I have varied my treatment, obtaining good results some years and less good in others. In two seasons I attempted rapid coseasonal desensitization, with inoculations daily or twice daily or every other day, running the dose up rapidly to the usual top dose. The last year I have reverted to my original method and have conclusively demonstrated its superiority. The general principles may be outlined as follows.

The principle of coseasonal desensitization is completely different from that of preseasonal treatment. In the latter the whole aim is to reach a high top or maximum dose. According to the theory of immunity this results in either the exhaustion of fixed antibodies attached to the cells or the compensatory production of so many free antibodies in the blood stream that the antigen as it enters the respiratory passages is neutralized by the free antibodies before it has an opportunity to combine with the fixed cellular antibodies. A high preseasonal dose is the desideratum.

In coseasonal treatment no such attempt is to be made and if it is made it results in failure. Frequent small dosage is the secret to success in coseasonal treatment. One starts in with a small dose, say 10 or 20 pollen units or 0.1 c.c. of 1/5,000 and gives daily inoculations. Improvement is usually almost instantaneous. I have seen it occur within thirty minutes. The dose is increased by 20 units each day until satisfactory relief results, and rarely is it necessary to exceed 80 units daily for the obtaining of 75 to 100 per cent relief. As soon as this is accomplished the interval between doses may be increased by one or two days. We then attempt to steadily increase the interval up to once or twice weekly. If symptoms return, doses are again given daily, and if they persist in spite of this the dose is cautiously increased.

In coseasonal therapy, keep the dose small to obtain maximum benefit, and give it as frequently as necessary to maintain the benefit. If you continue to push the dose up in spite of relief, you will reach a stage where the combined dose from your inoculation and from the inhalation of pollen is too great, and then the patient's symptoms will become worse instead of better.

Perennial Treatment.—The third method, perennial treatment, follows the completion of either preseasonal or coseasonal therapy. It is no small task to adequately desensitize a patient prior to the season. It seems poor logic, directly the season is over, to discard all of the advantage gained, allow the patient to again develop his sensitivity, and to have to repeat the entire process a year later. In administering perennial treatment the injections are continued slightly below the maximum dose, being given every two weeks throughout the year. Prior to the next season the task of rapid desensitization need not be repeated. It will only be necessary to step up the dose slightly, to the usual maximum. For example, with a top dose of 20,000 units just prior to the season we will drop down as soon as the season commences to a maintenance dose of 2,000 or 3,000 units every two weeks through the season and on through the year. A short time before the next season this will again be stepped up, in the course of four or five treatments to the usual preseasonal maximum of 20,000 units or 1 c.c. of 1/50 concentration.

The advantages of perennial treatment which was first described by Stewart and by Brown are (a) better results (b) avoidance of the inconvenience and labor to both patient and doctor of intensive preseasonal desensitization, (c) diminished tendency to anaphylactic reactions, (d) distribution of the doctor's work throughout the year, (e) the possibility of permanent immunity after three or more years of such treatment, (f) treatment may be started at any time of year.

In both preseasonal and perennial treatment it is well to drop from the maximum to the maintenance dose immediately with the onset of the season.

Anaphylactic Reactions.—A word about prevention and control of anaphylactic reactions. This is the *bete noire* of allergic therapy. And yet it is easily avoided and almost invariably promptly controllable. Except in the most fulminating reaction, the symptoms consist in urticaria, violent sneezing, or asthma coming on within a variable period after inoculation. A reaction of any great severity will occur within the first half hour or forty-five minutes. Reactions may occur after this but are usually easily controlled with ephedrin. If therefore the patient remains in the office thirty to forty-five minutes he can safely leave, provided he understands the symptoms of reaction and that he is to take ephedrin in the event any of these symptoms appear. He should understand that he is to take his ephedrin capsule and report back at once to the office.

Reactions at any time are usually promptly controlled by the methods outlined by Duke and Insley. Immediately upon the appearance of symptoms a tourniquet is placed upon the arm above the site of inoculation and 0.3 c.c. of adrenalin is introduced into the other arm. Symptoms are usually promptly relieved and the patient rapidly experiences adrenalin symptoms. The tourniquet is loosened and tightened at intervals over a period of half an hour to an hour

or two, until it can finally be left off permanently without return of symptoms. All of those who attempt desensitization therapy should read the articles by Duke and Insley in the original.

As a precautionary measure, when any treatments are given during the season, we always administer 0.3 c.c. of a mixture of equal parts of 1/1,000 adrenalin and 3 per cent ephedrin in the same syringe with the allergen extract.

RESULTS

The steady improvement in allergic therapy is exemplified in the report of Cooke, Vander Veer and Spain in which they have compared their results from year to year. In 1915 they obtained excellent results in 4 per cent. Eleven years later they obtained excellent results in 39 per cent. Their good results for 1915 total 47 per cent and in 1926 50 per cent. Their poor results in 1915 total 31 per cent and in 1926 11 per cent. No improvement was obtained in 15 per cent in 1915 as contrasted with only 3 per cent in 1926. Gay has concluded that on the average, desensitization therapy reduces the period of seasonal discomfort from a month and a half to a week and a half.

TABLE II
RESULTS 1931

RELIEF (%)	PERENNIAL No.	PERENNIAL %	PRESEASONAL No.	PRESEASONAL %	COSEASONAL No.	COSEASONAL %
100	12	46.3	1	7.6	6	40.0
90	6	23.1	5	38.5	3	20.0
75	6	23.1	5	38.5	4	26.7
50	2	7.5	2	15.4	2	13.3
Satisfactory 75-100%		92.5		84.6		86.7

TABLE III
RESULTS 1930 AND 1931

METHOD	SATISFACTORY RESULTS (75-100% relief)
Coseasonal 30 cases	73.3%
Preseasonal 34 cases	79.4%
Perennial 47 cases	93.6%

All allergists have had similar experience. Comparable improvement has been achieved in each of the three common methods of treatment. In my own experience the four outstanding recent advances have been (a) increase in the maximum preseasonal dose, (b) the development of control tests, (c) frequent and small coseasonal dosage, and (d) perennial treatment. Undoubtedly perennial treatment has in my experience been productive of best results. Up until this year I have felt that preseasonal treatment was superior to coseasonal. In 1930, 100 per cent of our patients obtained satisfactory relief following perennial desensitization. Seventy-one per cent obtained comparable relief from presea-

sonal treatment. Sixty per cent were adequately relieved from coseasonal treatment. This year, however, with the establishment of the principle of small dosage, our coseasonal results have been as good as our preseasonal. Either method, should, however, probably be followed by perennial therapy.

CONCLUSION

Twenty years have now elapsed since desensitization to pollens was first undertaken by Noon and Freeman. Results, at first mediocre, have been gradually improved year by year until at last we have reached that stage where, provided the experience and principles now available are properly applied, one may proceed with treatment, secure in the knowledge that his patient will receive with very rare exceptions from 75 to 100 per cent relief from symptoms. This is truly a remarkable achievement and represents the results of collaborative effort of a large group of earnest students of allergy.

We have almost reached that stage where we can modify the ancient dictum, in saying "nothing is certain in the future except death and taxes."

808 Professional Building.

OBSERVATIONS ON THE ACCURACY OF THE RABBIT OVULATION TEST FOR PREGNANCY*†

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CHIEF among the requisites of a good clinical test we must list accuracy, simplicity, and rapidity. Thus, in considering the various hormone tests for pregnancy, we find that Friedman's modification of the Aschheim-Zondek test[1] is apparently the most rapid, since results are obtained within forty-eight hours. It is also extremely simple, many investigators using but one injection of urine into a rabbit. In addition, a macroscopic examination of the ovaries for corpora hemorrhagica suffices, thus obviating the necessity of histologic preparations. The accuracy of this method, however, has not yet been fully established. Out of over 1000 tests reported in the literature we find approximately ten errors, resulting in an accuracy of over 99 per cent. Even at that some of these errors were undoubtedly due to various technical mistakes. This is superior to the accuracy of from 96 per cent to 98 per cent for the Aschheim-Zondek test, as reported by White and Severance.[2]

Our investigation was begun in June, 1931, with the idea of using the rabbit ovulation test only in those cases in which the diagnosis of a possible pregnancy was imperative, but doubtful, according to the usual means of examination. This, we believed, might constitute a more rigorous and valuable test of the method,

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than would the indiscriminate application of it to cases easily diagnosed by other procedures.

Table I shows the different technics and results of various investigators.

TABLE I

	AMOUNT INJECTED	TIME	NO. CASES	ERRORS
1. Friedman and Lapham[3]	4 c.c. t.i.d. for 2 days	48 hr.	92	0
2. Schneider[4a, 4b]	5 - 7 c.c.	24-30 hr.	200	2
3. Reinhart and Scott[5a, 5b]	5 c.c.	24 hr.	150	2
4. Martins[6]	5 c.c.	48 hr.	100	0
5. Magath and Randall[7]	7 - 15 c.c.	30 hr.	85	1
6. Bradford and Todd[8]	10 - 12 c.c.	24 hr.	22	0
7. Davis and Walker[9]	10 c.c.	24 hr.	65	2
8. Beasley[10]	?	?	52	0
9. Wilson and Corner[11]	5 c.c.	16 hr.	196	1
10. White and Severance[2]	10 c.c.	48 hr.	40	1
11. Ware and Main	10 c.c.	48 hr.	100	1
(Results here reported)			1102	10

The age and weight of the rabbits are of no particular consequence so long as the animal is mature, since some breeds are much larger than others, and may mature at different ages. The rabbits we use are virgin female chinchillas, five months of age. They are obtained from a breeding farm, so that all are of the same strain, age, and weight, and have been completely isolated from males for several months. Of late we have kept these rabbits in individual cages a week prior to use, in order to prevent possible formation of corpora hemorrhagica through mechanical stimulation of the genitalia. The urine injected was the first specimen voided in the morning. Samples collected from pregnant patients at other times during the day often give weak positives, probably due to a lack of concentration of the hormones. No aseptic precautions were taken, but unless the urine was to be used within a few hours, it was kept on ice. Nevertheless, several specimens which had been kept at room temperature for several days still produced good results.

During the first 20 tests we used two injections of urine of 10 c.c. each, into the marginal ear vein. The second injection was made twenty-four hours after the first, and the rabbit killed forty-eight hours following the first injection. This method may be advisable in cases of very early pregnancy, where the hormones are not concentrated, but in order to simplify the test as much as possible, for the rest of the investigation we used but one injection of 10 c.c., killing the animal in forty-eight hours. This happens to be exactly the same method as used by White and Severance.[2] Many investigators examine the test animal at the end of twenty-four hours or less. In our experience, however, this has not proved long enough. Occasionally, wishing to obtain a result quickly, we have injected two rabbits with the one specimen of urine, killing one rabbit in twenty-four hours, so that should the results appear doubtful, we could kill the second animal in forty-eight hours. In two cases among these, the twenty-four-hour rabbits gave doubtful findings, the follicles being very large, but quite clear. Both forty-eight-hour

rabbits gave the typical results of a positive test. This makes us feel that twenty-four hours is not always sufficient time, in spite of the excellent results of Wilson and Corner,[11] who allow but sixteen hours to elapse before examination. Occasionally we have allowed seventy-two hours to elapse, without any detriment to the results.

Besides the many corpora hemorrhagica found in the ovaries of rabbits injected with pregnancy urine, we have noticed that the uterus is also much enlarged, and generally very hyperemic, in comparison to the small white uterus usually present in negative cases. This difference is so striking that in the majority of the cases a diagnosis may be made when the uterus is exposed, and before examining the ovaries. This sign has proved of value in three cases of apparent spontaneous formation of corpora hemorrhagica of rabbits in which urine from nonpregnant patients had been injected. As indicated by Wilson and Corner,[11] there is not much difficulty in the differentiation of these false positives, by the fact that the corpora hemorrhagica are a dull red and the follicles are rounded, rather than conical. In these three cases, there were only from one to three corpora hemorrhagica present, and the uterus was small and pale. Occasionally, but not often, nonpregnant urine will cause enlargement of the uterus, or pregnant urine may not produce uterine enlargement. This uterine hypertrophy is more noticeable in virgin females than in those which have had young. Another advantage in using virgin rabbits is that their ovaries are small and clean, no traces of degenerating corpora lutea being present, so that results are unmistakable.

The only error we have found was a negative result following injection of urine from a patient found to be pregnant at operation. Urine collected a few hours following operation gave an excellent positive reading, as did specimens for two days following the hysterectomy. Since the first urine sample had been collected according to the ordinary ward routine, there is of course a possibility that an interchange with a specimen from another patient had occurred. It seems highly improbable that a specimen collected prior to operation should give a negative test, since those following hysterectomy gave positive readings.

A comparatively large number of injected rabbits died, possibly because of the so-called toxic urines. Out of over 170 rabbits injected, fifteen died within ten minutes to twenty-four hours following injection. In no case was this due to cloudy or alkaline urine, for all cloudy specimens were filtered, and although we do not routinely test the acidity of the urine, this was done in every case following the death of the rabbit. In none of these was an alkaline urine found. Five of these deaths were caused by urine from a case of hydatidiform mole and four from a patient with chronic nephritis and following a therapeutic abortion. The other deaths were scattered, but occurred during the extremely hot weather of the past summer, and may have been in part due to the high temperature. We have not tried the detoxification of such urines by extraction with ether, as recommended by Zondek.[13] Martins[6] believes that such a procedure actually increases the sensitivity of the test.

The explanation of the formation of corpora hemorrhagica in the ovaries, following injection of urine from a pregnant patient, is that these results are now believed to be due to the production of hormones by the placental tissue, and their subsequent excretion in the urine. Among these hormones are found two which

closely simulate those produced by the anterior lobe of the pituitary. These cause maturation of the follicles, ovulation, formation of corpora hemorrhagica and later corpora lutea. Indirect proof that the placenta may serve as a source of hormones, has been offered by several investigators. Collip[13] has demonstrated that human placental tissue contains large quantities of hormones. Others[14] have found that following castration of pregnant women, the urine at term contained at least the female sex hormone. This apparently proves that other tissues, presumably placental, were able to secrete this hormone in the complete absence of the ovaries. Again, Wilson and Corner[11] found that in cases of incomplete abortion, positive results with the rabbit test are obtained so long as living placental tissue remains. Both the hydatidiform mole and chorionic epithelioma also apparently produce these hormones, since positive results with ovulation tests are obtained in these cases. In fact, Ehrhardt[15] reports that the diagnosis of hydatidiform mole may be made because of the extremely large amounts of hormones excreted. This evidence, although indirect, leads one to believe that the placenta actively forms these substances rather than merely serving as a storage place for them.

Snyder and Wislocki[16] report that urine from pregnant animals such as the monkey, rat, cat, and dog, do not give a positive rabbit ovulation test.

The excretion of these hormones in the urine may be due to the huge quantities present in the blood. Ehrhardt[15] transfused nonpregnant women with 500 c.c. of whole blood obtained from pregnant women and demonstrated the presence of hormones in the recipients' urine with the Aschheim-Zondek test.

CLINICAL REPORT

We have tested specimens from 150 patients, and have followed 100 of these patients until the presence, or absence of pregnancy could be proved. A total of 135 specimens of urine have been tested on these completed cases.

One hundred patients were examined because pregnancy was questionable. This group may be subdivided into two groups.

a. Sixty-three patients who submitted 87 specimens and had a similar number of tests completed. All were positive.

b. Thirty-seven patients who submitted 48 specimens and had a similar number of tests completed. All were negative.

Analysis of positive group:

There were 4 cases of early pregnancy giving positive results.

The first was examined thirty days after her last menstrual period or six days after the patient expected to menstruate.

The second was examined thirty-five days after her last menstrual period or seven days after she expected to menstruate.

The third was examined forty-two days after her last menstrual period or twelve days after she expected to menstruate.

The fourth was examined forty days after her last menstrual period or twelve days after she expected to menstruate.

One case, Mrs. O. M., had missed three periods. She was diagnosed as a case of hydatidiform mole, and gave a positive test. Three days later she passed a part of the mole and was curetted lightly. Following the curettement fourteen tests were made over a period of two months. Five rabbits died in from ten minutes to twenty-four hours after injection and cannot be reported as test animals. Five rabbits gave positive tests before operation. A hysterectomy was done on this patient two months after she passed the mole, and specimens

tested daily. The test was positive on a specimen collected twenty-four hours after operation and negative on a specimen of urine collected forty-eight hours after the hysterectomy (Table II).

TABLE II

MRS. O. M., LAST MENSTRUAL PERIOD MAY 19, 1931. ADMITTED TO MEMORIAL HOSPITAL, AUGUST 4, 1931

DATE	RABBIT TESTS	RESULTS
Aug. 5	Rabbit injected	Positive
Aug. 11	Passed mole	—
Aug. 12	Curetted	—
Aug. 13	Rabbit injected	Positive
Aug. 14	Rabbit injected	Positive
Aug. 17	Rabbit injected	Died in 24 hours
Aug. 20	Rabbit injected	Died in 24 hours
Aug. 27	Rabbit injected	Died in 24 hours
Sept. 2	Rabbit injected	Died in 24 hours
Sept. 9	Rabbit injected	Died in 24 hours
Sept. 9	Curetted	—
Sept. 18	Rabbit injected	Positive
Oct. 4	Rabbit injected	Positive
Oct. 6	Rabbit injected	Positive
Oct. 10	Hysterectomy	—
Oct. 11	Rabbit injected	Positive
Oct. 12	Rabbit injected	Negative
Oct. 13	Rabbit injected	Negative
Oct. 14	Rabbit injected	Negative

Three patients with tubal pregnancies were tested and all gave positive tests. The earliest test was made six days after the menstrual period was due. These three patients were operated upon, and the evidence of tubal pregnancy was positive. Two of these cases were proved to be tubal pregnancies by microscopic examination of the tubes. One case at the time of operation was clinically a ruptured tubal pregnancy but was not studied further.

Two patients with multiple pregnancies gave positive tests before delivery.

Two patients, one with hypertension and the other with preeclamptic toxemia, also gave positive readings before delivery. In one of these cases the pregnancy was interrupted at the second month, by abdominal hysterotomy under spinal anesthesia, and every rabbit injected after the abortion, died almost immediately, even with a decreased dose of urine. Evidently the toxicity of the urine increased decidedly after termination of the pregnancy. The remaining cases in this group were later proved to be normal pregnancies.

The group which gave negative tests was composed of 37 patients and may be divided as follows:

2 patients with ovarian cysts.
3 patients with tuboovarian abscesses.
1 patient with tubercular peritonitis.
2 patients with fibroids of the uterus.
1 patient with a missed abortion.
2 patients forty-one years old later confirmed as cases of delayed menstruation, probably because of the menopause.

One patient, reported as a missed abortion, gave a negative test. She had missed four menstrual periods and had been clinically diagnosed as pregnant.

During the six weeks prior to the time her urine was tested her uterus had not enlarged. Two days after the specimen of urine was tested this patient passed a macerated placenta and fetus. The uterus was curetted but no normal healthy placental tissue was found.

This case further substantiates the finding of Wilson and Corner[11] who reported two cases of missed abortions. Positive tests were obtained in both during early pregnancy, and negative ovulation tests were produced by both cases before the uteri were emptied. A few days later macerated placentas were obtained in each case. Reinhardt and Scott[5] also reported one case of missed abortion which gave a negative test. Autopsy on this case revealed a detached and macerated placenta.

Two patients thought to be pregnant gave negative tests, and were proved at the time of operation to have uterine fibroids with no evidence of pregnancy. These cases give further evidence that uterine fibroids do not give a positive test, and help to substantiate the conclusions of Davis and Walker[9] that the positive test obtained by them from a patient with uterine fibroids was a technical error.

The remaining patients in this group were complete abortions.

Five patients formerly reported in the positive group deserve further mention as they were tested after delivery. The earliest negative reaction was obtained twenty-four hours after delivery, and the latest seventy-two hours after delivery. These results correspond with the findings of Wilson and Corner[11] who made daily tests on 16 patients after delivery.

SUMMARY

We have tested 150 patients for suspected pregnancy with the rabbit ovulation test, and later established the diagnosis clinically in 100 of these. This test has proved to be accurate with but one possible exception. The number of cases now reported in the literature is over 1000, with an accuracy of over 99 per cent.

We have found the rabbit ovulation test to be of particular advantage in the diagnosis of hydatidiform mole, missed abortion, ectopic pregnancies, uterine fibroids, menopause, and tuboovarian abscesses. The earliest pregnancy giving a positive result was six days after the menstrual period was due. Following delivery a negative result was obtained in from twenty-four to seventy-two hours.

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SEDIMENTATION RATES OF THE ERYTHROCYTES IN GENERAL DISEASE*

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SINCE the initial publication by Robin Fahraeus on the "Suspension Stability of the Blood," some ten years ago, numerous other articles have appeared and the determination of the sedimentation rate of the erythrocytes has become one of the more usual laboratory tests. The simplicity of this test, and the striking clean-cut positiveness, or negativeness, of the technical results, making it a test easily read and recorded, with a tendency to definitely fall into upper or lower registers, make it one of the useful tests in the hands of the clinical pathologist.

The clinical interpretation of the results is another matter. All the information on this is not yet at hand. Scattered reports on special diseases have been made but the test in general diagnosis has not yet been completely evaluated.

The test has been used in tuberculosis and is of considerable value, when correlated with other blood findings. In acute appendicitis, a disease where early diagnosis is essential, the test does not add especially to our diagnostic acumen. Adams Ray[1] showed that it was not of great value in the diagnosis of cancer and our results substantiate his work. In the early cases the rate may be normal or slightly increased, and in the late cases it may be only slightly pathologic. The accompanying circumstances, the presence or absence of infection or ulceration, the extent of tissue involved, and the situation of the tumor, apparently have much more to do with the sedimentation velocity than the mere presence or absence of a malignant process.

The estimation of this rate, technically, is accomplished by one of two methods; using a standard time and measuring the distance of the settling cells, or by using a standard distance and noting the variable time. The first is the method of Fahraeus and Westergren. The second is generally called that of Linzenmeir. In this series, we have preferred the second method, although they have been checked against each other and are perfectly comparable.[2]

Briefly the technic is as follows: a small Linzenmeir test tube, graduated at 1 c.c. and being about 50 mm. long, graduated as well at 6, 12, 18, and 24 mm. from

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the 1 c.c. mark is used. Two-tenths cubic centimeter of a fresh 5 per cent solution of sodium citrate is drawn into a sterile "insulin" syringe, and 0.8 c.c. of blood is added directly from the patient. The resulting 1 c.c. is placed directly in the test tube, without undue agitation, and the time noted. The number of minutes required for the red cells to settle down to the 18 mm. mark and leave the clear

TABLE I

DIAGNOSIS	NO. OF CASES	SEDIMENTATION TIME IN MINUTES		
		HIGH	LOW	AVERAGE
Normals				
Male	5	302	130	254
Female	8	414	120	226
Neurasthenia	6	361	125	224
Acute rheumatic fever	5	39	5	22
Atrophic arthritis	16	33	9	21.8
Osteoarthritis	5	49	40	44.5
Renal or ureteral calculi	4	377	109	262.2
Cystitis	17	70	13	27.5
Pyelitis	3	18	8	13.1
Pernicious anemia	4	39	11	28.7
Pelvic abscess	10	50	8	24.1
Localized infections	19	140	12	40.2
Septicemia	7	33	8	18.6
Phlebitis	3	55	9	24.3
Tuberculosis, active	10	31	6	16.1
Tuberculosis, healed	3	316	125	194.2
Lobar pneumonia	7	81	15	35.3
Bronchopneumonia	6	78	11	35.7
Acute bronchitis	5	82	14	45
Chronic bronchitis	3	83	17	47.3
Influenza	5	197	29	66.2
Diabetes	11	340	51	186.2
Diabetes with gangrene	4	47	27	35.5
Chronic cardiac valvular disease	13	434	60	198
Acute cardiac disease	7	187	21	48.7
Appendicitis acute				
Preoperative	1	129	129	129
Postoperative	9	260	24	59.2
Appendicitis chronic				
Preoperative	4	205	40	116.5
Postoperative	9	260	5	110
Tumors				
Nonmalignant	9	148	11	62
Malignant	26	221	7	52.2
Pregnancy				
Pernicious vomiting	2			
Incomplete abortion	3	42	19	30.5
Premature delivery	1	113	63	89
Toxemia, eclampsia	3	31	31	31
Normal postpartum	4	51	35	42
Hernia, pre- and postoperative	15	136	98	115.7
Fractures	31	310	26	121.2
Cholecystitis, chronic	3	158	8	66.3
Cholecystitis, acute	3	230	66	139
Catarrhal jaundice	2	32	17	24.7
Cirrhosis	2	310	70	190
Arteriosclerosis	8	22	11	16.5
Hypertrophy of prostate	9	278	20	116
		200	13	108
Total	348			

plasma above is called the sedimentation time. As is shown in Table I, this will vary from a few minutes, in the more acute cases, to several hours in the normal individual.

This condition is thought to be[3] one of the fundamental responses of the body to disease and comparable to fever or leucocytosis. The mechanism is probably a relative increase in the globulin and fibrinogen, with a decrease in albumin in the serum. This changes the surface tension and the viscosity of the serum though the physical-chemical relationship is doubtlessly complex and may involve other factors.

Clinically, an increasing sedimentation time means that the patient is improving, and conversely, a diminishing sedimentation time means that the patient is getting worse. A short time (a rapid rate) indicates the presence of some severe inflammatory condition; a longer one the absence of inflammation.

To gain an adequate idea of the value of this test, we ran a series on 348 of our patients, with the results shown in Table I and Figs. 1, 2, and 3. These were gen-

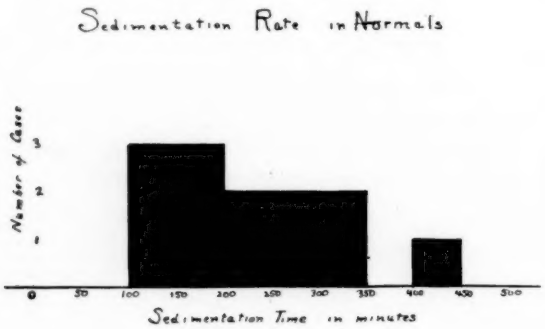

Fig. 1.

eral hospital patients, both medical and surgical, and include a wide variety of clinical conditions.

First on the list is a group of 13 normals, nurses, technicians, and doctors, or those hospital patients admitted for observation and showing no symptoms or signs of disease. These were all over two hours, extended up to six hours, with an average of around four. In our experience this has been quite uniform, and any rate under two hours, or especially under one hour, leads to further study to ascertain the type and extent of organic disease.

Then we studied an interesting group of patients with joint disease. Five cases of acute rheumatic fever showed an average sedimentation time of 22 minutes. This agrees with the work of Ernstene[4] who found that the sedimentation rate in acute rheumatic fever showed an even greater relative increase than the leucocyte count, and that this index remained positive several days to several weeks after the white count had become normal. The test became positive very quickly after the individual clinically showed swollen joints and was positive even in cases where the white blood count was low.

A group of 16 cases, in which the clinical diagnosis of atrophic arthritis had been made, had an average time of 21.8 minutes, varying in the relatively narrow limits of 9 to 33 minutes. Five osteoarthritis varied from 40 to 49 minutes, with

an average of 44.5. This agrees with the work of Dawson, Sia and Boots[5] from the Presbyterian Hospital in New York, in which they found that the sedimentation rate of the red cells was parallel to the severity and extent of the process, though in cases of the so-called osteoarthritis, the sedimentation rate, while as a rule, is increased, rarely attains values obtained in the other type of the disease. They report a list of 196 cases.

A third group of 6 in which the clinical diagnosis of neurasthenia was made showed times varying from 125 to 361 minutes, with an average of 224. We do not say that these patients had no physical disease, but at least there were no other definite, direct clinical signs of it.

Increased familiarity with this test, therefore, has caused us to place increased reliance on it in separating these groups. We are all familiar with the

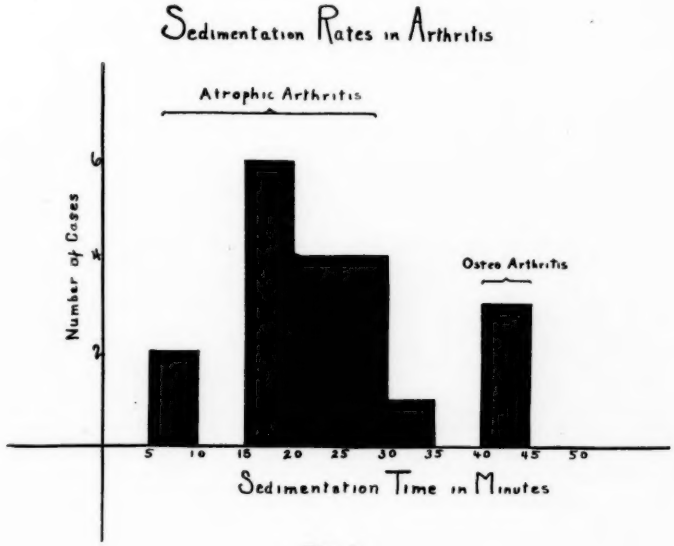

Fig. 2.

clinical picture of the patient who complains of pain in the shoulder, hip or arm, and in whom ordinary physical examination, leucocyte and differential count, x-ray, etc., may show no abnormality. Has this patient an early arthritis without physical signs, or is he having pain without physical basis? The question frequently is raised in compensation cases and the physician on the witness stand hesitates to commit himself too far in either direction. It is in just this group of cases that the cell sedimentation time has proved of great value.

Following up those cases with a short sedimentation time (an increased rate) without other physical signs, we have found in our series that sooner or later they show definite signs of joint involvement, which may or may not improve under treatment. And the group with normal sedimentation rates have, in our experience, shown no further clinical signs of organic disease. We feel that the test is of distinct value in this differential diagnosis.

Another group, in which the test is of differentiating value is in urologic conditions. The average time, we have found to be greatly shortened in pyelitis

(13 minutes), and in cystitis (27.5 minutes), but normal in cases of renal or ureteral calculi without infection (262.2 minutes). Even in the early cases of pyelitis, without prostration or any considerable amount of pyuria, the sedimentation time has been distinctly lowered. Seventeen cases of cystitis varied from 13 to 70 minutes, all of them in a low range, averaging 27.5 minutes. Nine cases of hypertrophy of the prostate varied from 13 to 200 minutes, varying obviously with the amount of infection in the bladder, accompanying the primary clinical condition.

Chronic or severe acute infections, in general, gave a markedly reduced time; e. g., pelvic abscess, ten cases with an average of 24.1 minutes; septicemia, seven cases, with an average time of 18.6 minutes; phlebitis, three cases with an average time of 24.3 minutes; other localized infections, 19 cases, with an average of 40 minutes.

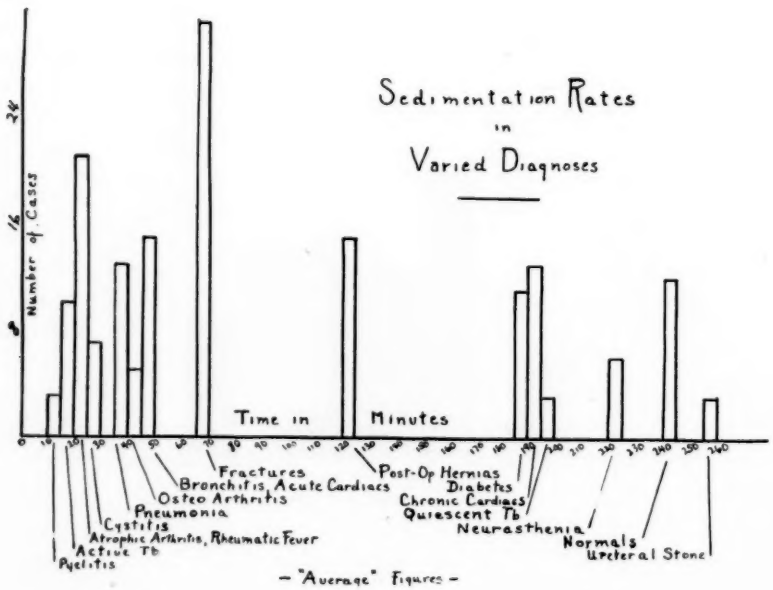

Fig. 3.

The test has been used in tuberculosis with outstanding results. We had ten proved active cases of tuberculosis, with sedimentation times varying from 6 to 31 minutes, with an average of 16.1 minutes. Three cases of quiescent or healed tuberculosis varied from 125 to 316 minutes, with an average of 194, that is, about normal.

Thirteen cases of lobar and bronchopneumonia gave cell sedimentation times averaging at 35 minutes. Acute and chronic bronchitis lowered the time to a slightly less degree, as did influenza.

Eleven cases of diabetes varied from 51 to 340 minutes, obviously depending on concurrent factors, for 4 cases with gangrene had reduced times averaging 35 minutes.

Cardiac conditions varied with the type of disease, or conversely the test can be used to separate the acute endocarditis, from that disease where symptoms are

due to old, chronic scarring of the valves. Thirteen cases of chronic valvular heart disease had an average time of 198 minutes, while 7 cases of acute, infective cases averaged at 48.7 minutes. How often we wonder if an old cardiac lesion, with obvious valvular disturbance, has engrafted onto it an acute process, with infection of the endocardium? This test may help us to decide about this point.

The test in cases with tumors varied within wide limits; in the nonmalignant group from 11 to 148 minutes, with an average of 62; and in the 26 malignant cases, from 7 to 221 minutes. This suggests that the test is of less importance in the diagnosis of cancer but is influenced by infection and other extraneous factors.

Fractures generally showed a somewhat reduced time, more sharply so in compound fractures, but present to a less extent in even simple fractures, and gradually returning to normal as the bone healed.

A number of other conditions are listed in the table, where the cases were too few, or the results too variable to draw accurate conclusions. We realize that each of these groups of cases is small and that the conclusions on the "average" figure from such a small group cannot be held too mathematically rigid.

CONCLUSIONS

The red cell sedimentation rate is a test that is easily performed and the results easily read.

Normal individuals have a sedimentation time (Linzenmeir method) of over two hours.

Severe acute and chronic inflammatory conditions greatly lower this time.

This is of diagnostic and prognostic value in rheumatic fever, atrophic and osteoarthritis and in differentiating mild degrees of these conditions from functional disturbances, symptomatically similar.

The test is also of value in separating inflammatory from noninflammatory conditions of the urinary tract, and is of considerable confirmatory evidence in many other conditions.

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STUDIES IN VENOUS PRESSURE*

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I. HISTORICAL

EARLY descriptions of methods of measuring venous pressure in man were published by Frey,[1] von Basch Gaertner[2] and others. Von Recklinghausen[3] describes the method upon which the instrument by Hooker and Eyster[4] was based for estimations applicable to clinical work. They reported venous pressure in a series of cardiac cases with the use of this instrument. Determinations with the direct method were first made by Moritz and Tabora,[5] Schott[6] and others. These observations compared closely with those found by indirect methods. Many workers have studied venous pressure in relation to its various physical and physiologic aspects. During the past few years, the work of Hooker, Eyster, Middleton and several others has increased the interest in venous pressure and shown its value in clinical medicine. Clark[7] was the first to make a close study of venous pressure in cardiac decompensation and its relationship to prognosis and treatment.

II. PHYSICAL MECHANISM CAUSING VENOUS PRESSURE

With the body at rest, the pressure of the blood in the arteries is fairly constant, and the same holds true with regard to the veins. As the heart beats, blood is taken from the veins and forced into the arteries, and differences in pressure in the arteries and veins develop, due to resistance offered to the moving stream by the arterioles and capillaries. Physical conditions cause the pressure to be highest at the driving head, that is, the heart, and to fall increasingly towards the end of the system, or the inflow into the heart. The term "peripheral resistance"[8] has been used to explain the marked fall of pressure occurring between the arterial and venous portions of the vascular system. Thus, the two obvious factors: the head of pressure or cardiac output and the variable peripheral resistance, explain the change of pressure in the two sides of the vascular system.

A factor which must be further considered is the peripheral vascular bed, that is, the arterioles and capillaries. It has been shown[9] that abolition of vasoconstrictor control is followed by a marked fall in arterial pressure, but little or no change is noted in venous pressure. Removal of vasoconstrictor tonus, by greatly reducing peripheral resistance, theoretically should cause a marked rise in venous pressure, but this is compensated for by an increase in the total vascular bed. Splanchnic stimulation causes contraction of the arterioles and capillaries, i. e., a reduction of the vascular bed. This is followed by a slight rise in venous pressure.

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In ordinary conditions the pressure in any superficial vein is due to two factors. The first of these is the influence exerted by the pumping action of the heart, the second the effect of gravity. This latter factor is modified by the influence of skeletal muscular contraction which decreases the caliber of the veins and expresses the blood from them; hence, the venous pressure is reduced. These factors are largely eliminated by having the subject at complete body rest, in the recumbent position, and the vein selected for pressure determination on a level with the entrance to the right heart.

III. METHODS FOR DETERMINING VENOUS PRESSURE

Two method have been used for determination of venous pressure, the direct and the indirect methods. In the first, a trocar is introduced into the vein and from this a tube extends upward. As the blood comes to a height in the tube the reading is taken in centimeters. This method is probably the more accurate, but for clinical purposes when determinations are made daily or more often, it is less useful than the indirect method which has found greatest favor in clinical work. This method depends upon the principle that if outside pressure is exerted upon a superficial vein, the vein will either be patent or collapsed, depending upon whether the outside pressure is lower or higher than that of the blood within the vein. Many forms of instruments for determining venous pressure by the indirect method have been constructed. Probably the best is the one originally designed by Hooker and Eyster[4] and which has been constructed by J. S. Hipple, Mechanician of the Medical School of the University of Wisconsin, from whom an instrument was obtained for my use.

IV. NORMAL VENOUS PRESSURE AND PHYSICAL CONDITIONS WHICH AFFECT IT

In the determination of venous pressure the patient is at rest in the recumbent posture. In a group of patients without circulatory disturbances it was found that the average venous pressure was 60 mm. of water, the extremes being 35 mm. and 90 mm. Many variations have been given for normal venous pressure with determinations made under the same basic conditions. Frey,[1] the first to use the indirect method, found the extremes in sitting position to be 54 mm. to 179 mm. of water. Hooker[10] reported readings varying from 30 mm. to 150 mm. of water. Runge[11] found pressures between 48 and 66 mm. of water. Readings on numerous normal subjects in the recumbent position by Eyster and Middleton[12] were found to range between 50 mm. and 60 mm. of water, which has been accepted as the normal.

Many factors may influence normal venous pressure. The most important of these has been shown to be posture, which causes a rise when the patient changes from the recumbent to the erect posture. It was found that the pressure rises on an average of 20 mm. of water from recumbent to the erect position, ranging from 70 mm. to 90 mm. in the series studied by me.

Muscular activity has been found to be a factor which influences venous pressure. If an estimation is taken (a) with the patient at rest and (b) after he has had a brisk walk, it will be found to increase on an average of 20 mm. to 40 mm. of water. Hooker[13] made observations during vigorous exercise and found an in-

crease of from 70 mm. to 140 mm. of water. Villaret, St. Girons and Grellity-Bosviel[14] also show an increase after a prolonged walk.

The influence of respiratory activity is significant. The negative pressure in the thorax during inspiration facilitates the flow of blood into the heart and causes a rise of pressure in the intrathoracic veins. The varying negative pressures in the thorax, associated with different phases of respiration, produce correspondingly varying effects on venous pressure.[15] This will be the greater, the deeper the respiration. During quiet breathing in man it has been found that so far as the pressure in the peripheral veins is concerned, there is little variation between the phases of inspiration and expiration. This is due to absorption of the differences encountered in the thorax by the inertia of the blood column between the central and peripheral veins. Definite respiratory variations in pressure in a peripheral vein are absent or else very slight during quiet breathing.[15] This condition has also been shown to be true by Bedford and Wright[16] and Waud.[17] No variations have been found in the cases which have been studied in this series. In hyperpnea, respiratory variations became evident and there is a fall of mean venous pressure in the normal subject of 7 mm. to 30 mm. of water.[16] In cardiac decompensation the overventilation tends to lower venous pressure, but even with this factor the pressure is found high.

V. VENOUS PRESSURE IN CARDIAC DECOMPENSATION

This is the one condition in which the majority of workers agree that consistent changes in venous pressure occur. Frey[1] in 1902, stated that a rise in venous pressure may be an early sign of impending cardiac failure. Gaertner[18] one year later, noted pressures as high as 230 mm. of water in several cases of cardiac decompensation. Hooker and Eyster[4] in 1908, reported several cases of decompensation in which venous pressure was at first high, falling to normal as compensation was reestablished. Clark[7] in 1915, showed that: (1) peripheral venous pressure rises to an abnormal level in this condition; (2) the degree of rise is approximately proportional to the degree of clinical cardiac failure; (3) that such determinations are of value in prognosis and treatment. Many workers have shown similar results. Villaret, St. Girons and Grellity-Bosviel,[14] using the same method, found an abnormally high venous pressure in decompensation and state that a rising pressure may be an early sign of impending heart failure. Eyster and Middleton[12] in 1924, reported a series of cases in which the venous pressure maintained a high level during decompensation. Eyster[15] in 1929, showed a definite increase in venous pressure during decompensation with a reduction as compensation returned.

The mechanism of the increase of venous pressure during cardiac decompensation has been widely discussed. It was early recognized that venous pressure may be influenced by the rate at which the heart receives blood from the veins and forces it into the arterial system.[8] A reduction in cardiac output such as occurs in vagal inhibition would obviously tend to produce stagnation in the vena cava, and a rise in venous pressure would result. Increased cardiac output on the other hand would tend to cause a depletion of the venous blood and a low venous pressure. Thus, the cardiac output is a variable factor, which causes changes in

venous pressure. However, a different conception of the relation of venous pressure and cardiac output has developed in recent years. It sets forth the idea that venous pressure is probably the main factor in determining cardiac activity.

These two conceptions started a discussion which has not yet been fully closed. At present, it is the idea of the majority of observers that the two views are interdependent and that cardiac activity, since it is regulated by venous pressure, is a most important factor in tending to stabilize it. When the heart muscle becomes diseased and its power to contract is lessened, a decrease in cardiac output results. This leads to a stagnation of blood in the veins with a rise of venous pressure; edema and engorgement due to blood stasis, supervene. The kidneys show marked congestion, evidenced usually by reduction of urinary output and albuminuria. As congestion decreases, the urinary output increases. This was first shown by Clark,[7] and has been observed in this series also. Eyster[15] in his most recent work has shown that the influence upon the heart muscle due to lack of oxygen supply is of importance. There is less oxygen during decompensation due to the slowing of the pulmonary circulation which results in poor aeration. Thus, the blood in the coronary arteries does not have the proper oxygen supply. Dilatation of the heart results in contractions which are more rapid and less forceful. These produce a further reduction in cardiac activity with its lowered cardiac output and increased venous pressure.

Practically all observers are agreed that a definite increase in venous pressure occurs during decompensation. A series of 35 cases showing decompensation has been studied. It was found that the pressure was high with decompensation, and as compensation was restored, the venous pressure returned to normal. In cases showing steady or persistent increase in venous pressure, death usually occurred. In some of these cases it was found that the venous pressure would remain at a high level for some time and then a further rise would be noted before any definite change could be detected clinically. In patients whose venous pressure readings were of this type, the prognosis was usually grave. At the height of decompensation with its increased venous pressure, it was found that the urinary output was extremely small. As the venous pressure began decreasing, the urinary output became greater. In Table I, a few typical cases are shown.

VI. VENOUS PRESSURE IN CHRONIC HEART DISEASE

Various observers have shown that unless there is evidence of cardiac failure the venous pressure is normal. A group of 15 cases of organic heart disease who have never suffered decompensation has been studied. The venous pressure in these patients did not exceed normal limits.

The next group of cases of interest are those who have alternate decompensation and compensation. In a study of 15 of these cases during compensation the same results are found as in those who have never had decompensation.

Many of these patients continue to take a maintenance dose of digitalis daily and the amount of their physical activity is extremely small.

VII. VENOUS PRESSURE IN ARTERIAL HYPERTENSION, NEPHRITIS AND UREMIA

Hypertension has little effect on venous pressure. Fuch[19] found a venous pressure of 56 mm. of water in 6 cases of hypertension. Bedford and Wright[16]

TABLE I

CASE NO.	TYPE OF HEART DISEASE	VENOUS PRESSURE	HOSPITAL DAYS	CONDITION	RESULT
1	Syphilitic. Aortic insufficiency	140	8	Marked edema, poor	Died
2	Rheumatic. Mitral stenosis and insufficiency	110	23	Poor, edematous	Died
		115	to		
		135	30	Worse	
		150	32		
		140		No change	
		140	38		
		140			
		150	39	Worse, edematous	
		160	to		
		170	46	Much worse	
		190			
3	Rheumatic. Mitral stenosis and insufficiency, aortic insufficiency	120	19	Poor, edematous	Died
		100	23	Improved	
		90	23	Improved	
			28		
		110	32	Slightly worse	
			to		
		90	38	Improved	
		140	60	Worse, marked edema	
			to		
			70		
		160	80	Very edematous	
4	Arteriosclerotic and hypertensive	120	10	Poor, very edematous	Compensated
		125	to		
		110	15		
		120	25	Unchanged	
		110	to		
		90	40	Improved	
		90	60	No edema but not completely compensated	
5	Arteriosclerotic	160	2	Very poor	Compensated
		170	5	Worse	
		130	10	Much improved	
		110	15	Improved	
		90	20	Improved	
		70	25	Improved	
6	Arteriosclerotic and hypertensive. Auricular fibrillation	130	2	Fairly good Moderate edema	Compensated
		120	5	Improved	
		90	10	Improved	
		80	14	Compensated	
7	Arteriosclerotic. Auricular fibrillation	110	31	Edema, fair	Compensated
		110	34	Edema, fair	
		100	38	Improved	
		90	42	Compensated	
		80	46	Compensated	
8	Infectious with cardiac hypertrophy and dilatation	140	6	Moderate edema, fair	Compensated
		110	10	Improved	
		80	12	Compensated	
		60	14	Good	
9	Arteriosclerotic and thyroid. Auricular fibrillation	180	1	Extremis, edema 4+	Compensated
		160	3	Slightly improved, poor	
		140	5	Improved, edema 2+	
		130	8	Improved, edema 2+	
		120	12	Improved, edema +	
		120	14	Improved, edema slight	
		120	16	Improved, edema slight	
		100	18	Good, no edema	
		90	20		
		80	21	Fine, no edema and compensated	

TABLE I (CONTINUED)

CASE NO.	TYPE OF HEART DISEASE	VENOUS PRESSURE	HOSPITAL DAYS	CONDITION	RESULT
10	Rheumatic, mitral stenosis, auricular fibrillation	120	3	Moderate edema, poor	Compensated
		100	5	Slight decrease in edema	
		90	10	Improved	
		90	12	No edema	
11	Arterioselerotic and hypertensive	160	1	Marked edema	Compensated
		140	4	Slight improvement	
		120	8	Improved	
		100	10	Improved	
		80	14	Compensated	
12	Syphilitic, aortic insufficiency	130	4	Poor, very marked edema	Died
		120	7	Improved	
		100	8	Improved	
		90	10	Good, no edema	
	Patient developed auricular tachycardia	100	12	Slightly worse	
		120	13	Much worse	
		140	14	Edema	

TABLE II

		VENOUS PRESSURE
1	Rheumatic heart disease. Mitral stenosis and aortic stenosis and insufficiency	90
2	Rheumatic heart disease. Aortic insufficiency with mitral insufficiency	80
3	Rheumatic heart disease. Mitral stenosis	80
4	Rheumatic heart disease. Mitral stenosis	70
5	Syphilitic heart disease. Aortic insufficiency	90
6	Rheumatic heart disease. Mitral stenosis	80

have also found normal pressure. In our group of 10 cases of arterial hypertension without evidence of heart failure, it has been found that there is only a slight increase above the average normal. Thus, we believe we can say that in this type of vascular lesion venous pressure shows little or no change. Types of these cases are shown in Table IV.

It has been possible to observe several patients with marked hypertension, severe nephritis, and uremia. These patients had edema of the lower extremities but the clinical picture was not that of cardiac failure. Only 4 such cases have as yet been followed through, so no conclusions are made. No explanation is offered other than to suggest the possibility that with decrease of kidney function and appearance of edema the results may be comparable to those seen in cardiac failure. The venous pressure was high when first taken and increased until the patient died.

TABLE III

TYPE OF HEART DISEASE	VENOUS PRESSURE
Syphilitic	
1	80
2	80
Arteriosclerotic and hypertensive	
1	80
2	60
3	80
4	80
5	90
6	70
7	80
8	80
9	80
Rheumatic	
1	80
2	70
3	90
4	70

TABLE IV

TYPE OF DISEASE	VENOUS PRESSURE	BLOOD PRESSURE
Arteriosclerosis and hypertension Chronic nephritis	80	190/100
Arteriosclerosis with chronic nephritis	80	200/100
Arteriosclerosis and hypertension	90	210/100
Arteriosclerosis Chronic nephritis	90	200/135

TABLE V

TYPE OF DISEASE	VENOUS PRESSURE	BLOOD PRESSURE	RESULT
General vascular sclerosis. Chronic nephritis	150	250/150	Died
General vascular sclerosis. Chronic nephritis	140 to 180	180/120	Died
General vascular sclerosis. Chronic nephritis	140 to 170	210/160	Died
General vascular sclerosis. Chronic nephritis	120 to 180	190/120	Died

VIII. VENOUS PRESSURE IN LOBAR PNEUMONIA

One of the diseases most frequently complicated with circulatory failure is pneumonia. It is found that lobar pneumonia has no effect on venous pressure until circulatory failure occurs. However, it is shown that by frequent determina-

tions the appearance of failure can be foretold by a rise in venous pressure before it is evident clinically. Eyster[15] says that as long as the heart is sufficient and a normal circulation is maintained, the venous pressure remains normal. There is a rise in pressure as in cardiac decompensation when the heart fails. Increase of venous pressure is the first indication of failing heart. Eyster and Middleton[12] reported several cases of lobar pneumonia of which 6 showed a venous pressure within normal limits. The other cases showed an increased venous pressure and evidences of cardiac failure. Fuchs,[19] in 9 cases of lobar pneumonia, showed 8 to have normal venous pressure. Two of these patients died within thirty-six hours without further determinations being made. The other case had a high venous pressure and died. Kastlin and MacLachlan,[20] in a series of 90 cases of lobar and bronchial pneumonia, showed that a majority of these patients with normal venous pressure recovered, the mortality being 14 per cent. The majority of their patients with slightly increased venous pressure recovered, the mortality being 11 per cent. The majority (61 per cent) of cases with high venous pressure died. The cases showed very little evidence of circulatory failure except increased venous pressure. This series agrees with the previous observers, that an increase of venous pressure in pneumonia is evidence of circulatory failure and such determinations are very valuable in prognosis, as the usual signs of cardiac failure are not seen at the time when the pressure begins to rise.

IX. THERAPY IN VENOUS PRESSURE

It is extremely difficult to separate the influence upon venous pressure in cardiac decompensation of drug therapy, bed rest and limitation of diet and foods. No drug is known which in the normal animal will act as a specific to reduce venous pressure.[21] Digitalis and caffeine used in normal animals showed a rise in arterial pressure but no change in venous pressure. Morphine and strychnine caused no change.[21] Nitrites caused a fall. In man, inhalation of amyl nitrite caused a slight increase.[16] The use of digitalis has long been known to be of value in cases of cardiac decompensation. Its action is to decrease the rate and increase the force of the beat. If we consider the conception of heart rate as a factor in venous pressure, we must believe that digitalis is of value in decompensation by reducing rate, increasing force of driving head, reducing venous pressure and causing the signs of decompensation to disappear. In a group of cases of cardiac decompensation studied, a combination of drugs has been used. It is, of course, impossible to say that any one thing is responsible for the results. It is believed that digitalis is of value. Every case studied received this drug in some form. It was found that in a few cases of abnormal rhythm, auricular fibrillation, tachycardia, etc., the signs of decompensation returned unless the maintenance dose of digitalis was continued for some time.

Diuretics of some form were used in most of these patients. The results from theocin and theocalcin were practically the same. Salyrgan was found to be quite valuable in the reduction of edema, with a consequent decrease in venous pressure. It was found to be of more value and somewhat less apt to cause bad effects than novasurol. The best treatment for decompensation was a combination of bed rest, morphine, limited diet and fluids, digitalis, and later salyrgan. The majority of the patients recovered compensation.

Venesection in the treatment of cardiac decompensation has long been used. Clark[7] used this method and showed that after venesection the venous pressure was reduced but that it rose again very rapidly. Eyster[15] has shown that high venous pressure was reduced definitely after venesection for a long period of time. If the load of increased pressure on the heart can be reduced for a short time, the factors causing decompensation may be abated and compensation reestablished rapidly. In some of the cases reported by Eyster[15] there was definite improvement and subsequent recovery. Others died within forty-eight hours, showing no improvement. The greater number of the cases showed improvement and for this reason it is believed that venesection is of definite value in the treatment of cardiac decompensation.

X. SUMMARY AND CONCLUSIONS

In this study of venous pressure several factors have been discussed. From these, certain conclusions may be drawn. The physiologic mechanism affecting venous pressure has been summarized. The factors which affect venous pressure in normal individuals, the changes of venous pressure in cardiac disease and associated conditions have been listed. It has been shown that the normal venous pressure taken under basic conditions as described is about 60 mm. of water. In organic heart disease unless there is evidence of failure, the venous pressure does not exceed the normal limits. As cardiac failure appears, it may be indicated by a rise in venous pressure before other clinical evidence is present. A continued high venous pressure or a rising pressure during treatment is a bad prognostic sign. High arterial pressure alone causes little or no change in venous pressure. With chronic nephritis and uremia the venous pressure is increased. In pneumonia, unless cardiac failure occurs, venous pressure does not show an increase. An increase in venous pressure in this disease is the first evidence of cardiac failure. Reduction of venous pressure in cardiac decompensation and the restoration of compensation cannot be attributed to any one therapeutic measure but to the combination of several.

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THE SPECIFIC GRAVITY OF THE BLOOD DURING SPINAL ANESTHESIA*

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THE following is a report of the specific gravity of the blood during spinal anesthesia. The determinations were made by the delicately accurate falling drop technic of Barbour and Hamilton[1]: "*Principle:* A drop of blood (or other body fluid) of definite size is released below the surface of a nonmiscible mixture. Its rate of fall depends on its density, which can be easily calculated as soon as the rate of fall of a similar drop of standard solution of known density (released under identical conditions) is available for comparison."

A total of twenty anesthesias were studied. Twelve only serve as a basis of this report; there was one death shortly after the administration of the spinal anesthetic, five others were supplemented by gas or ether and in two no control readings were obtained. Thus these twelve cases, carefully worked up, represent smooth anesthesias from beginning to end, in which blood specific gravity determinations were made before and during the operations. However, it may be stated here that eighteen of the cases followed the same general pattern described below.

These observations were gathered over a period beginning October, 1926, and ending in March, 1928. We had contemplated working up a larger series but various things prevented this. However, we feel that these few cases were carefully controlled and are worthy of presentation. The perspective obtained by the elapsed time has aided us in the proper evaluation of our findings.

General Analysis.—Table I represents the manner in which our data were collected. There were three general patterns discernible and these are depicted in graph form in Charts 1, 2, and 3. Table II shows at a glance the composite findings in the nine cases not graphed.

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*From the Gynecologic Service, Barnert Hospital.
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Attention is invited to the falling time of the drop of blood in that it was found to be a more sensitive indicator of changes in blood density than when the falling time was interpolated into terms of specific gravity. To one not accustomed to determining the specific gravity of the blood, a change of one point in the third decimal place may seem insignificant, but to the person clocking off the falling time on a stop watch split to tenths of a second, a change of one to twenty seconds is quite impressive. Normally the specific gravity of the blood from moment to moment is so constant that a change of even one point in the third decimal place must be considered as heralding a definite, though slight and transitory, change in the status of the blood. To make the tables more comprehensible the nearest half second of falling time was used. Likewise, only the second and third decimal values (to the nearest half of the fourth decimal value) of the specific gravity of the blood are used. Thus a reading of 1.0532 is represented by figure 53; a reading of 10535 is represented by the figure 54.

TABLE I

BLOOD DENSITY AND BLOOD TENSION DURING SPINAL ANESTHESIA*

TIME A.M. BP	TIME A.M. BD	TENSION	FALLING TIME BLOOD	FALLING TIME STANDARD	BLOOD DENSITY
9:57		104/72	17.7	18.6	10560
10:03	10:05	88/64	20.4		10533
	10:09		21.9		10529
10:14	10:16	84/62	21.7	18.5	10531
	10:25		23.0		10511
10:28	10:29	84/60	20.8		10527
10:35	10:36	84/64	20.9	18.4	10526
10:45	10:46	86/64	20.6		10529
	10:57		20.5		10530
10:58	10:59	154/92	20.4		10531
11:06	11:07	182/98	19.9		10535
11:08	Operation ended				

*Case 1, October 1, 1926. Female, aged fifty. Vaginal repair. Control (prior to operation) B. P. 172/78, B. D. 10552.

a. Pattern I, Chart 1.—In a previous publication, one of us[2] made preliminary mention of the specific gravity of the blood during spinal anesthesia. Table I is a reproduction of the protocol published at that time. The statement was made then "that with the fall in blood tension that follows the administration of the spinal anesthetic there is a concomitant fall in blood density, it being noted that as the patient begins to react from the anesthesia the rise in blood tension is followed by an increase in blood density." We have had no reason to change this comment since this is what we are pleased to term the "usual or general pattern" obtained. One fact may be noted here, to be discussed later, is that in Cases 1, 10, and 19, there is a preliminary rise in blood density during the first five minutes of the anesthesia, even though the blood pressure has begun to fall.

b. Pattern II, Chart 2.—In this pattern there was no immediate transitory increase in blood density. There were, rather immediate wide and sweeping fluctuations downward and upward, the general trend being consistently parallel with the change in blood tension.

TABLE II.

Composite Findings in Nine Cases of Spinal Anesthesia.

Case 3.				Case 4.				Case 6.				Case 8.				Case 12.				Case 14.				Case 15.				Case 17.				Case 19.				
Bl. Pr.		Sp. G. Bld.	F.T. Bld.	Bl. Pr.		Sp. G. Bld.	F.T. Bld.	Bl. Pr.		Sp. G. Bld.	F.T. Bld.	Bl. Pr.		Sp. G. Bld.	F.T. Bld.	Bl. Pr.		Sp. G. Bld.	F.T. Bld.	Bl. Pr.		Sp. G. Bld.	F.T. Bld.	Bl. Pr.		Sp. G. Bld.	F.T. Bld.	Bl. Pr.		Sp. G. Bld.	F.T. Bld.	Bl. Pr.		Sp. G. Bld.	F.T. Bld.	
S.	D.			S.	D.			S.	D.			S.	D.			S.	D.			S.	D.			S.	D.			S.	D.			S.	D.			
78	56	50	25	134	78	47	32	100	66	49	36	108	62	51	26	114	62	58	20	116	74	55	21	114	84	56	19	170	90	55	23	110	70	50	22	Control Readings.
84	66	...	...	128	72	45	41	108	68	49	32	70	54	46	50	102	38	58	19	68	44	55	20	76	56	55	21	90	76	49	39	114	74	51	22	
64	?	49	27	106	66	45	39	104	70	50	32	88	54	51	28	84	54	58	19	82	50	53	23	74	56	55	20	80	60	54	24	104	70	50	22	
34	?	46	37	94	50	44	46	90	66	49	35	80	56	50	30	76	52	56	22	78	50	53	23	62	48	53	23	84	64	55	22	98	68	49	24	Interval between readings ranged from three to eight minutes.
?	?	48	30	96	48	45	43	90	60	49	35	86	58	50	30	78	58	56	21	80	50	54	22	60	44	51	27	76	60	56	21	88	68	49	25	
?	?	46	36	98	63	45	45	88	60	48	39	84	62	49	32	82	54	56	21	80	50	54	22	63	36	52	24	82	60	54	25	76	58	48	26	
?	?	50	25	90	50	45	42	100	72	48	32	84	64	49	34	88	60	56	22	96	60	56	20	51	36	54	22	76	60	55	23	82	58	49	24	
?	?	50	25	98	58	45	42	...	...	48	41	84	60	50	30	92	64	57	21	102	68	56	20	50	38	53	22	78	60	56	22	86	60	50	23	
58	52	50	25	122	78	45	43	80	58	48	38	88	60	49	34	92	60	58	20	106	66	55	20	70	50	53	23	74	60	54	24	92	64	49	25	
...	...	...	...	...	...	46	39	84	62	49	35	90	64	46	50	102	68	58	20	108	68	56	20	104	74	56	20	80	64	55	23	98	66	50	23	
...	...	...	...	...	...	46	38	90	63	49	36	...	...	47	43	102	68	59	19	...	...	...	...	114	88	56	20	94	76	56	22	96	66	49	24	
...	...	...	...	...	...	...	...	88	64	48	41	82	64	49	33	108	76	59	19	...	...	...	...	112	70	56	19	102	78	56	21	...	...	...	...	Blood for specific gravity values taken from capillary beds in finger tips.
...	...	...	...	...	...	...	...	92	68	47	43	...	...	...	...	114	80	59	18	...	...	...	...	110	70	57	18	...	...	...	...	...	...	...	...	
...	...	...	...	...	...	...	...	96	70	48	41	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	
...	...	...	...	...	...	...	...	100	74	47	47	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	
...	...	...	...	...	...	...	...	104	70	48	41	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	
...	...	...	...	...	...	...	...	108	78	48	37	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	
Spinal Anes. Admin. 2:35 p. m., Oct. 8, 1926.				Spinal Anes. Admin. 4:56 p. m., Oct. 8, 1926.				Spinal Anes. Admin. 3:20 p. m., Nov. 5, 1926.				Spinal Anes. Admin. 2:40 p. m., Nov. 24, 1926.				Spinal Anes. Admin. 11:35 a. m., Oct. 2, 1927.				Spinal Anes. Admin. 11:52 a. m., Oct. 27, 1927.				Spinal Anes. Admin. 10:45 a. m., Oct. 28, 1927.				Spinal Anes. Admin. 11:30 a. m., Jan. 4, 1928.				Spinal Anes. Admin. 2:23 p. m., Mar. 13, 1928.				
Operation ended 3:32 p. m.				Hysterectomy. Operation ended 5:55 p. m.				Perineorrhaphy and Interposition.				Perineorrhaphy, Appendectomy, and Gilliam Suspension.				Perineorrhaphy and Interposition.				Appendectomy. Operation ended 12:40 p. m.				Laparotomy and Vaginal.				Vaginal Hysterectomy.				Repair of Vesico-Vaginal Fistula.				
								Operation ended 4:35 p. m.				Operation ended at 3:38 p. m.				Operation ended at 12:33 p. m.				Operation ended 12:40 p. m.				Operation ended at 11:59 a. m.				Operation ended at 12:27 p. m.				Operation ended at 3:11 p. m.				

Bl. Pr.=Blood press.
S=Systolic
D=Diastolic
F. T.=FallingT

c. Pattern III, Chart 3.—In two of the twelve cases the blood pressure sounds were inaudible. These were recorded as zero pressures. Babcock[3] in 1922 stated that "a fall in the systolic pressure in the radials to zero is occasionally seen." Of course, the radial systolic pressure cannot actually fall to zero, since such zero pressures are incompatible with life. In the one death mentioned, the blood pressure

Chart 1.—Case 1, Oct. 1, 1926, female, aged fifty, vaginal repair. Spinal anesthesia, administration at 9:50 A.M.

fell to zero and the exceedingly high specific gravity of the blood of 1.067 was obtained. The blood densities in these two cases followed the usual downward trend, but on the recovery of the blood tension the blood density was inclined to fluctuate in steeper gradients upward.

In general, then, it was found that the greatest fall in systolic pressure took place in five to twenty-two minutes after the administration of the anesthetic; the greatest fall in diastolic pressure in five to twenty-six minutes; the greatest fall in the specific gravity of the blood in fourteen to thirty-three minutes.

As an additional comment on blood pressure and blood density in general, one gains an increasing respect for the constancy of the diastolic pressure and the latter's possible immediate effect upon the blood density.

Note also was made of the fact that in seven cases vomiting or eructations took place at the depths of blood tension and blood density.

Chart 2.—Case 9, March 18, 1927, female, aged fifty-five, perineorrhaphy. Spinal anesthesia, administration at 9:24 A.M.

DISCUSSION

Perhaps the only value these observations may have is that they throw a small additional light on the question of shock. No human experimental method so closely simulates the condition of shock as does spinal anesthesia. This is especially true in that the latter and true shock have one great thing in common, namely, the extreme hypotension. The surprisingly good clinical condition of patients under spinal anesthesia is comparable to the work of Sherrington (cited by Macleod[4]) on spinal shock in monkeys. Sherrington found that in a monkey whose spinal cord

had been cut far forward, the posterior part of the animal was in shock, there was hypotension, while the monkey amused himself by catching flies with his fore limbs.

Any discussion of blood density presupposes and accepts the idea that any change in the blood is at once manifested by a change in its specific gravity. Such a change may be brought about by an alteration in the number of erythrocytes.

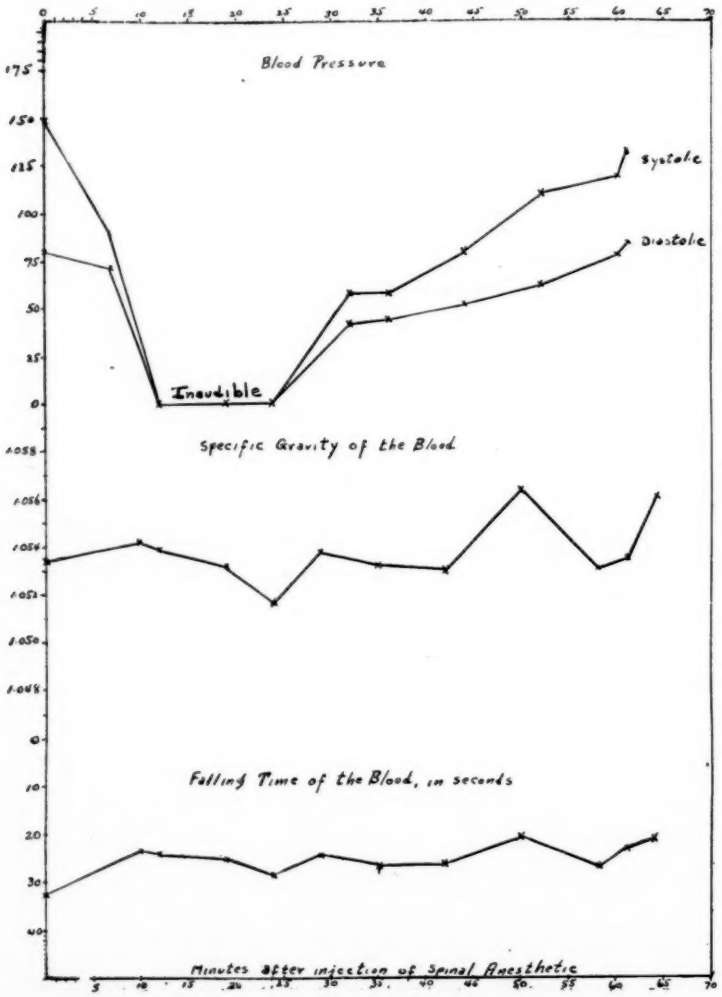

Chart 3.—Case 10, female, aged fifty, perineorrhaphy, October, 1927. Spinal anesthesia, administration at 10:12 A.M.

leucocytes, platelets, by alteration of the hemoglobin content, fibrinogen content, ash content, by shifting water balances (viscosity); by alteration of lipoid values. Since this work shows that the blood density is consistently lowered during spinal anesthesia and since it is thought that a greater blood pressure is required to propel more viscid than less viscid blood, we are of the opinion that the lowered blood density is a protective mechanism. The lowered blood density is a response to the lowered blood pressure which is thus better able to propel the blood through the body. It is recognized that the reverse may be the case.

The transitory rise in blood density at the beginning of some of the spinal anesthesias may be due to a concentration of the blood, a jamming back of blood in the capillaries. A concentration of the blood in this manner is said to precede the condition of true shock.

As one studies the tables and charts it is discernible that the end stages of the anesthesia are ushered in by a wave-like change in blood density. This is a definite phenomenon which the less sensitive mechanism of blood pressure does not always record. One gets the feeling that the patient is rising out of the depths of anesthesia in undulating fashion, and not in the easy gradual way which a picture of the blood pressure alone would indicate.

SUMMARY

1. The results of blood density determinations in twelve cases of spinal anesthesias are recorded and an attempt made to analyze them.

2. It appears that three general patterns are obtainable with the dominant finding that of parallelism of blood density with blood tension. A lowered blood tension is accompanied by a lowered blood density.

3. The suggestion is advanced that this lowered blood density is a protective mechanism.

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ETIOLOGIC STUDIES IN HODGKIN'S DISEASE*

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THE nature and the etiology of Hodgkin's disease are still in doubt. Bacteriologic studies have given divergent results which have been interpreted variously. Sternberg[1] attributed it to a modified or attenuated form of the tubercle bacillus. Reed[2] described the histologic picture in the nodes and called attention to the frequent association of tuberculosis and Hodgkin's disease sometimes even to be found in the same node. Fraenkel and Much[3] described a granular, nonacid fast bacillus seen in sections from the nodes. Negri and Mieremet,[4] and independently Bunting,[5] cultivated a diphtheroid bacillus from Hodgkin's tissue. Bunting and Yates[6] inoculated monkeys with these organisms. The blood picture and lymphoid involvement in these resembled Hodgkin's disease. Many workers have cultivated diphtheroid organisms from Hodgkin's lymph nodes (Billings and Rosenow,[7] Kusunaki,[8] Litterer,[9] Lanford,[10] Olitsky,[11] Rhea and Falconer,[12] Ives,[13] Mel-

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lon,[14] Bloomfield,[15] Torrey,[16] and Steel[17]). Mellon, in a bacteriologic study of seven strains of organisms isolated from Hodgkin's nodes demonstrated that they differed from each other in staining, vigor of growth, resistance to antiformin, involutionary forms, pigment formation, and other biologic characteristics. Similar organisms have been cultivated from the lymph nodes in conditions other than Hodgkin's disease (Lanford, Yates, Bunting and Kristjanson,[18] Harris and Wade,[19] Steel). Torrey showed that different strains of diphtheroids could be cultivated from the same lymph node of Hodgkin's disease. Bloomfield announced a bacterial flora for normal and diseased nodes. Cunningham[20] and Fox[21] believed that no one organism with definite morphologic and cultural characteristics had been isolated from Hodgkin's nodes. Agglutination and complement fixation tests using organisms isolated from Hodgkin's nodes and the serum from patients with the disease have been practically negative (Kristjanson,[22] Eberson,[23] Moore,[24] Torrey, Bloomfield, Olitsky and Lanford). Administration of a vaccine made from the organisms isolated has no more curative effects than injection of foreign protein (Billings and Rosenow, Mellon, Moore, Wallhauser and Whitehead,[25] Litterer and McVey and Wilson[26]). While lesions similar to Hodgkin's disease have been reported in lower animals, (MacFadyeon,[27] Hodgson,[28] Simons,[29] Reid,[30] Yates,[31] Simonds,[32] Jobling[33]) Rolleston[34] is of the opinion that more definite evidence from histologic examination is necessary before the occurrence of Hodgkin's disease in animals can be accepted. Nonconfirmatory reports have followed the attempt to produce the disease in horses, monkeys, rabbits, guina pigs, and chimpanzees (Reed, Lanford, Rhea and Falconer, Mellon, Bloomfield, Torrey, Moore, Cunningham and McAlpin,[35] Stewart and Dobson[36] and DeLeon and Reyes[37]). L'Esperance[38] reported the production of the disease in chickens by the intravenous injection of macerated lymph node, and believed it to be due to the avian tubercle bacillus. Twort[39] was unable consistently to demonstrate a specific animal or vegetable parasite in the affected tissue from Hodgkin's disease, either by direct microscopic examination, cultivation or animal inoculation (rabbits and guinea pigs). Nor did his assortment of in vivo or in vitro experiments give any positive results. His researches extended over a period of six years and included very elaborate studies on 61 cases. Warthin[40] considered Hodgkin's disease to be a malignancy. More recently Medlar[41] has suggested that it is a neoplasm originating in bone marrow.

It was with the hope of securing additional evidence that the present experimental studies were undertaken. Lymph nodes from five cases presenting the clinical features of Hodgkin's disease were used. The diagnosis was confirmed by microscopic examination. They contained typical lymphadenoma giant cells, eosinophiles, plasma cells, and endothelial overgrowth, fibrosis and mitotic figures. Two of the nodes were obtained as biopsy specimens, and three at postmortem examination. To avoid extraneous contaminants the surfaces of the nodes were first seared with a hot spatula. Then with sterile instruments a small piece of tissue was removed from the center through the seared surface.

Bits of this, about 0.1 gm. in weight, were crushed and planted in brain broth, dextrose broth, hormone broth, melted plain agar, brain broth in deep tubes, blood agar, ascitic fluid, and broth and agar enriched with ascitic fluid. Cultures were incubated for thirty days before discarding. The cultures from one case remained sterile. In one case there was a diphtheroid bacillus and a motile bacillus and a

variety of other organisms which were regarded as contaminants. From three cases a variety of diphtheroid bacilli were isolated. An identical organism occurring in each of the three cases was made the subject of further study. They were apparently identical in all particulars. This organism was markedly pleomorphic, showed curved granular rods 2-4 μ in length and coccoid forms 0.6 μ in length, V-shaped, dumbbell and bottle forms were seen, but no banded forms. It stained well with Loeffler's methylene blue. By Gram's method the granules stained positively but the matrix varied in its retention of the dye. It was nonacid fast and nonmotile. In the cultures it required ten to fourteen days to make its appearance. A low oxygen tension was required for its growth which was obtained by planting the tissue at the bottom of a deep culture tube filled with media, or by the use of sodium hydroxide and pyrogallic acid. After several months' cultivation this preference for low oxygen tension gradually disappeared and the organism became completely aerobic. The colonies in agar were small, round, grayish white, mainly discrete, measuring 0.5 to 2 mm. in diameter. Daughter colonies forming at right angles to the stab appeared rapidly after twenty to thirty days' incubation. These were larger, less sharply circumscribed and less opaque than the earlier ones. In the shallow broth cultures grown under partial anaerobic conditions there developed a clouding of the lower third of the media and a heavy sediment at the bottom. No acid or gas was formed on mannite, galactose, saccharose, glucose, levulose, maltose or lactose. It did not acidify or coagulate litmus milk, produced no indol, no pigment nor did it liquefy gelatin. Bloomfield,[15] Torry,[16] and others have described a similar organism isolated both from normal and from diseased lymph nodes. The latter gave it the name Bacillus lymphophilus because of the frequency with which he encountered it in lymph nodes.

Following the technics outlined by Hiss and Zinsser[44] agglutination, complement fixation, bacteriolytic and precipitation tests were made using this organism and serum from patients with Hodgkin's disease and from normal controls. No demonstrable agglutinins, precipitins, complement fixing bodies or bacteriotropic substances were demonstrated. For the complement fixation tests the blood serum was obtained from three patients with Hodgkin's disease in whom the Wassermann reactions were negative. The control sera used were from three normal individuals with negative Wassermann's and from three syphilitics with positive Wassermanns. No fixation of complement with antigen prepared from the organism in question occurred with any of these sera. For bacteriotropic substances, the blood serum of patients with Hodgkin's disease was added to cultures of the organism. Also Berkefeld filtrates of Hodgkin's lymph nodes were added to the cultures with negative results.

Animals were inoculated in some cases with the organism just described; in other cases with bits of lymph nodes aseptically obtained. The organism was introduced in a variety of ways. Following the method described by Moon and Konzelmann[42] cotton plugs saturated with a heavy suspension of living organisms were implanted under aseptic precautions through a trocar into the peritoneal cavity and into the subcutaneous tissue. Bits of sterilized bone were allowed to incubate in the cultures for several days and were then implanted as were the cotton plugs. Salt solution suspensions of the organism, broth and soft agar cultures of it were injected intravenously, intraperitoneally, into and beneath the skin. The

animals were carefully watched for clinical manifestations. Routine temperature readings and blood counts were made. Weights were checked frequently. The postmortem examinations of the animals were made with aseptic precautions. Postmortem bacteriologic studies of the lymph nodes, spleen, liver, kidney, blood and foci of infection were made routinely. Specimens from the lymph nodes and visceral organs were fixed in 10 per cent formalin and Zenker's fluid, cut and stained with hematoxylin and eosin and also with carbol fuchsin in certain cases.

Monkey (macacus rhesus) No. 1 received a total of three intraperitoneal inoculations of the organism, two subcutaneous implantations in the cervical region, of bone impregnated with the organism, and implantations in the neck of fresh lymph node substance. Following subcutaneous inoculations the regional lymph nodes became slightly swollen but rapidly subsided. Bi-weekly blood counts were within normal limits.[43] The blood sedimentation time averaged between 3 and 4.5 mm. in one hour. Temperature records and clinical data were normal. The animal was permitted to live for eleven months and was healthy when destroyed. Except for a chronic proliferative foreign body reaction about the cotton plugs and about the implanted bone, no lesion was demonstrated by gross or microscopic examination. Cultures from spleen, lymph nodes, kidney, and cotton plugs were sterile. A coccus was cultivated from the liver.

Monkey (macacus rhesus) No. 2 was inoculated intraperitoneally with the organism. Two and one-half months later he was killed with ether and a careful examination was made. There were no gross or microscopic lesions. The organism inoculated was isolated from a mesenteric lymph node and from the spleen.

Four half grown chickens of the Plymouth Rock variety were inoculated as follows: Two received subcutaneous implantations of bone impregnated with the organism cultivated from Hodgkin's nodes. These lost their appetites, lost weight and became anemic. One died one and a half months later, the other two and a half months later. At the postmortem examination no gross or microscopic lesion was demonstrated. The organism injected was not recovered. No acid fast organisms were demonstrated in any of the tissues. Two chickens were inoculated subcutaneously with pieces of fresh lymph node from Hodgkin's disease. Seven and a half months later they were healthy and were killed and examined. No gross or microscopic lesion was demonstrated. Sections from all the organs were examined but no acid fast organisms were found.

Two healthy half grown dogs were inoculated intraperitoneally with a heavy suspension of the organisms on cotton plugs introduced through a trocar. The dogs were observed for six months before they were destroyed. Clinically they showed no evidence of disease. Temperature and blood counts showed no significant variations. The blood sedimentation time was within normal limits. At the postmortem examination no gross nor microscopic changes were demonstrable in the lymphoid structures nor elsewhere. Cultures were made from the cotton plugs and from the various tissues. The inoculated organism was not recovered.

As a routine diagnostic procedure guinea pigs were inoculated with pieces of lymph nodes from each case. In addition five guinea pigs were injected intravenously and intraperitoneally with freshly isolated cultures. Preceding inoculation the animals had received intravenous and intraperitoneal injections of India ink for the purpose of blocking the reticulo-endothelial system and rendering the

animals perhaps more susceptible to infection. They lived from two to five months. At the postmortem examination no lesions of Hodgkin's disease nor of tuberculosis were found.

Sixteen rabbits were inoculated as follows: Crushed lymph nodes were implanted intraperitoneally on a cotton plug in two. In eleven the organism was introduced in the various ways described earlier in this article. In one the animal received an intravenous injection of India ink prior to the implantation of the organism. Two were inoculated with the substance of fresh Hodgkin's lymph nodes and cultures of the organism. The animals receiving large amounts of freshly isolated organism died in less than a month with progressive loss of weight, anorexia and paralysis of hind quarters. The others were destroyed six months following inoculation. There were no signs of Hodgkin's disease during life. At postmortem examination there were found in the tissues no lesions resembling Hodgkin's disease.

COMMENT

In four out of five cases of Hodgkin's disease studied, a variety of pleomorphic bacilli were cultivated from the lymph nodes. Experiments designed to demonstrate the relationship between one of these organisms present in each of three cases, and Hodgkin's disease were carried out. No agglutinins, precipitins or complement fixing bodies were found in the blood serum of patients suffering with Hodgkin's disease. Inoculation of this organism and the Hodgkin's lymph nodes into monkeys, guinea pigs, chickens, dogs and rabbits failed to reproduce the disease. Cultures made at the postmortem examination of the artificially produced foci of infection, the lymphoid structures and other visceral organs demonstrate that in some of the animals the organism persisted in the foci and spread to other organs; that in others it persisted only in the foci; while in still others no evidence of infection nor of persistence of the organism at the site of inoculation resulted

It is established that with appropriate technic bacilli of the genus corynebacterium can be isolated from Hodgkin's tissue. There is no sound experimental evidence that these organisms bear any etiologic relationship to the disease. The cultivation of similar organisms from pathologic lymph nodes, the seat of a variety of lesions, is further evidence against this relationship. The only significant association possible is that as in typhoid and leprosy they might be the cause of the disease but do not reproduce it in animals. Study by all other known methods does not confirm this.

Inoculation of pieces of fresh Hodgkin's nodes and of the organisms cultivated from them into chickens failed to produce either Hodgkin's disease or avian tuberculosis. Postmortem bacteriologic cultures and histologic examination of their tissues failed to reveal acid fast organisms. As it is difficult to get laboratory fowl which are free of avian tuberculosis it is probable that L'Esperance[38] mistook this common finding for significant experimental results. Branch[5] suggests that she was dealing, not with Hodgkin's disease, but with avian tubercle bacillus infection in the human being.

CONCLUSIONS

A variety of organisms of the genus corynebacterium can be cultivated from a high percentage of cases of Hodgkin's disease. An identical organism cultivated from three cases has been studied experimentally.

No precipitins, agglutinins, complement fixing bodies, or bacteriotropic substances for this organism were found in the blood serum of patients suffering with Hodgkin's disease.

Monkeys, chickens, dogs, guinea pigs, and rabbits were inoculated in a variety of ways with this organism and with pieces of fresh Hodgkin's lymph nodes. In none of them did the lesions of Hodgkin's disease develop.

Neither avian tuberculosis nor lesions resembling Hodgkin's disease were found in fowl inoculated with cultures or with fresh tissue from Hodgkin's disease.

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THE MAYFAIR HOUSE, LINCOLN DRIVE AND JOHNSON STREET.

THE EFFECTS OBSERVED FOLLOWING THE INTRAVENOUS AND SUBCUTANEOUS ADMINISTRATION OF FLUID. AN EXPERIMENTAL STUDY ON DOGS*

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THE common practice of forcing the administration of fluid without specifying either the amount or the time interval is inexact, and in certain instances, may be harmful. Attention has been directed to this fact by Lewis,[1] de Takats,[2] Hunt, McGann, Rowntree, Voegtlin,[3] and others.

Any method which would permit the clinician to determine accurately either a deficit or an excess of water in the tissues of a patient, from time to time, would be a desirable addition to modern methods of treatment.

The experimental work which forms the basis of this report was started for the purpose of finding such a method. We also wished to observe if large quantities of fluid produced harmful results, and if so under what conditions. The aims have not been realized as yet, but certain observations have been made which are significant.

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The literature concerning the blood changes in conditions affecting the body water balance is voluminous. Klein and Nonnenbruck[4] found that in subjects with normal metabolism intraduodenal infusion of water regularly caused a reduction in the number of erythrocytes in arterial blood. Boycott and Douglas[5] gave hypertonic glucose solution to normal and nephritic rabbits and found that the latter took a longer time to adjust themselves. Several deaths were attributed to cardiac failure which were said to occur more frequently in the nephritic than in the normal rabbits. Smith and Mendle[6] injected an amount of fluid, which they considered equal to the blood volume of a rabbit, and then by repeated blood counts and hemoglobin determinations, found that the bulk of the fluid had left the blood stream in the first five minutes. This fluid could not be accounted for by passage into the muscles or by edema. The fluid in the serous cavities and that in the stomach and intestines therefore as well as the volume of urine are concerned with the disposal of fluid leaving the circulation. There is considerable disagreement as to the fate of the fluid injected. Dastre and Loye[7] found that 75 per cent of the fluid injected intravenously passed into the serous cavities and tissue spaces. MacCallum[8] injected large volumes of fluid intravenously and from a cannula tied into the lumen of the intestine, obtained up to 15 per cent of the injected fluid.

Keith, Rowntree, and Geraghty[9] in a series of articles, the first published in 1915, introduced the dye method of determining blood volume. This has since been elaborated upon and values have been determined for numerous conditions, experimental and clinical. Lampson and Rosenthal[10] declare that true blood volume, plasma volume or red cell volume cannot be measured by any of our methods. They give reasons for the belief and conclude that it is impossible by the present methods to differentiate between blood changes due to fluid loss and those the result of vasomotor or circulatory changes. One of these authors in collaboration with others later stated that on account of the inability to determine the amount of red blood cells and fluid lost or gained, it is impossible to interpret the results obtained in terms of fluid exchange between blood and tissues even though 25 c.c. of normal salt solution is given per kilogram of body weight. They found that volumes determined by means of vital red and hemoglobin did not always agree.

METHODS

Well nourished, apparently normal, adult dogs were used for the experiment. Isotonic sodium chloride solution was administered subcutaneously in amounts of 2000 c.c. or intravenously in an amount of 1500 c.c. Observations on the following factors were made both before and after the administration of fluid. (1) Physical response; (2) blood volume changes; (3) blood cell counts; (4) hemoglobin concentration; (5) total blood proteins; (6) tissue thirst; (7) electrocardiograms; (8) urinary changes. These observations were repeated on animals in which renal damage had been produced, nephrectomies performed, or diphtheria toxin administered, for the purpose of producing cardiac damage.

Blood volumes were determined by the vital red method, as described by Hooper, Smith, Belt, and Whipple.[11]

Before any fluid was given the average blood volume of each experimental animal was established by two or more estimations at intervals of from five to ten days. Blood volume estimations were repeated immediately after intravenous administration of fluid and two hours after subcutaneous injection.

Total serum proteins were determined by means of the micro refractometric method, using the Zeiss dipping refractometer.

Skin wheals were produced by the introduction, intradermally, of 0.2 c.c. of normal salt solution and were used on a small series of dogs.

Electrocardiograms were run on a series of animals, before and after the administration of fluid.

Urinary volumes, specific gravities and the presence or absence of albumin were determined following the introduction of fluid.

To check the dye method of blood volume determination, two dogs were bled and perfused.

To produce myocardial damage, diphtheria toxin was given, following the method described by Stewart.[12] We found it necessary to reduce the dosage by half in order to keep the animals alive for a sufficient length of time to complete the observations. Renal damage was produced by clamping the vessels bilaterally as done by Ivy[13] and associates. Total nephrectomies were done on two dogs.

RESULTS ON NORMAL ANIMALS

Blood Volume.—The results obtained on the effect of the intravenous injection of 1500 c.c. of isotonic sodium chloride solution, during a period of fifteen to twenty minutes, on the circulating blood volume of dogs as determined by the vital red method are shown in Table I. No increase in circulating blood volume that could be measured by the dye method occurred.

Blood Counts and Hemoglobin Determinations.—It was found that five minutes after the intravenous administration of 1500 c.c. of 0.9 per cent sodium chloride solution, the red cell count and hemoglobin content were decreased significantly, the average decrease being 23 per cent. The maximum and minimum decrease varied in the different dogs from 10 to 36 per cent, but in the same dog from time to time the decrease did not vary more than 10 per cent.

TABLE I

SHOWING THE EFFECT OF THE ADMINISTRATION OF 1500 C.C. OF 0.9 PER CENT SODIUM CHLORIDE SOLUTION INTRAVENOUSLY ON BLOOD VOLUME DETERMINED BY THE DYE METHOD

DOG NO.	CONTROL BLOOD VOLUME DETERMINATIONS*		POST INJECTION BLOOD VOLUME DETERMINATIONS	
	NO. TRIALS	AV. BLOOD	NO. TRIALS	AV. BLOOD (C.C.)
4 15.7 Kilo.	5	1418	1	1397
5 11.8 Kilo.	5	1118	4	1128
6 10.5 Kilo.	2	1097	1	923
7 19 Kilo.	5	1367	2	1365
3 20.5 Kilo.	8	2117	1	2127

*Variations from time to time were less than ±100 c.c.

These results indicated a blood dilution following the injection of fluid, and were at variance with the results cited above. This apparent dilution occurred so uniformly that we questioned the accuracy of the dye method for determining total blood volume under the conditions of our experiment. Consequently, we checked the vital red method with the method of bleeding and perfusing in an animal to which no fluid had been given and in another after the administration of 1500 c.c. of isotonic salt solution. We found that the results obtained by the dye method checked to within 2 per cent in the normal animal but failed to check in the hydrated animal. The volume obtained by bleeding and perfusing was greater than when vital red was used for the determination. The value determined by bleeding and perfusing corresponded closely to the increase indicated by the blood count, hemoglobin and hematocrit determinations.

The injection of 2000 c.c. of isotonic sodium chloride solution subcutaneously had no effect on the red cell count two hours after the injection and before complete absorption had taken place.

Hematocrit Determinations.—Hematocrit readings five minutes after the intravenous administration of 1500 c.c. of fluid revealed an average dilution of nearly 20 per cent. Although there were variations of from 10 to 20 per cent in different animals the values in the individual animal remained fairly constant. The dilution persisted for at least one hour but had disappeared at the end of twenty-four hours.

Total Blood Proteins.—A study of the total blood proteins before and after the intravenous administration of isotonic salt solution shows that they were decreased from 20 to 52 per cent in the various dogs. In the same dog from time to time the maximal variation of the decrease was 12 per cent. In Table II are shown the results in which the total blood proteins were followed in three dogs up to one hour after the injection of fluid. From this table it will be seen that the total proteins do not return to the prefluid level within one hour.

TABLE II

SHOWING THE TOTAL BLOOD PROTEINS BEFORE AND AFTER THE INTRAVENOUS ADMINISTRATION OF 1500 C.C. OF 0.9 PER CENT SODIUM CHLORIDE SOLUTION

	DATE	PRE-FLUID VALUE	IMMEDIATELY FOLLOWING FLUID	15 MIN. LATER	1 HR. LATER	PER CENT DECREASE
Dog 5	12-11	7.3	4.3	5.6	5.6	39
Wt.	1-12	6.9	4.2	4.8	4.9	39
11.8 K	1-22	6.5	3.8	4.9	4.9	41
	3-2	6.5	3.1	4.1	4.5	52
Dog 4	12-17	6.7	4.9	5.8	5.8	36
Wt.	1-14	7.1	5.6		6.3	47
15.7 K						
Dog 7	12-3	7.1	5.6		6.3	21
Wt.	12-6	6.9	5.1	5.7	6.4	26
19 K	1-13	7.5	6.0	6.1	6.5	20

In Table III is shown typical data on three dogs in which all determinations were made as nearly simultaneously as possible. This was not always technically feasible. Three determinations on Dog 5 are shown to illustrate the variations en-

countered in the same dog. The only striking discrepancy is in the decrease in total proteins in the case of Dog 5, tests "A" and "C," in which the blood counts and hematocrit figures checked, but the decrease in total proteins is approximately twice what it should be on the basis of blood dilution alone. The only explanation of this discrepancy that occurs to us is that some protein has actually left the circulation, which does not usually happen.

Tissue Thirst.—Skin wheals were produced by introduction of 0.2 c.c. of 0.9 per cent sodium chloride solution intradermally. Several hundred readings were made at various times under varying conditions. From the data gathered no definite conclusions could be drawn due to the extreme variability of the length of time required for the wheal to flatten to the level of the surrounding skin.

Urine.—A large number of urinary outputs were studied. After the production of kidney damage and after the administration of diphtheria toxin, there was a one-plus albumin in the urine but this was reduced to a trace following the injection of fluid.

TABLE III

COMPOSITE CHART SHOWING THE CHANGES FOLLOWING THE INTRAVENOUS ADMINISTRATION OF 1500 C.C. OF ISOTONIC SODIUM CHLORIDE SOLUTION

		BLOOD VOLUMES	BLOOD COUNTS	TOTAL* PROTEINS	HEMATOCRIT**
Dog 4 Wt. 15.7 K	Prefluid	1418 c.c.	7,010,000	6.7	59
	Postfluid	1397 c.c.	6,240,000	5.8	65
	Per cent decrease		12	13	10
Test A Dog 5 Wt. 11.8 K	Prefluid	1118 c.c.	4,320,000	7.3	58.8
	Postfluid	1233 c.c.	3,350,000	4.3	77
	Per cent decrease		23	47	25
Test B Dog 5	Prefluid	1118 c.c.	7,920,000	6.9	
	Postfluid	1016 c.c.	5,120,000	4.2	
	Per cent decrease		35	39	
Test C Dog 5	Prefluid	1118 c.c.	6,300,000	6.5	60
	Postfluid	1016 c.c.	5,010,000	3.8	77
	Per cent decrease		21	41	23
Dog 7	Prefluid	1391 c.c.	7,040,000	7.5	58
	Postfluid	1548 c.c.	5,720,000	6.0	71
	Per cent decrease		18	20	19

*Percentage of total serum protein.
**Percentage of serum in whole blood.
Volumes listed as prefluid are the average of a series of normal volumes as shown in Table II.

There was a tendency toward lowered specific gravity in the dogs that had had the renal vessels clamped but the return to normal within twenty-four hours was just as regular as in normal dogs. This was true in animals given salt solution on three successive days.

Results on Animals Given Diphtheria Toxin.—Four dogs were given diphtheria toxin in an attempt to produce myocarditis. Observations were then made on these animals before and after the administration of fluid.

Blood Volumes.—It is evident from the results (Table IV) that diphtheria toxin tends to cause a decrease in blood volume which is partly, but not entirely accounted for by the loss of weight. In two of the three dogs given 1500 c.c. of salt solution there was an increase in blood volume following the first injection. Later however no increase resulted.

Blood Cell Counts, Total Protein and Hematocrit.—As will be seen from Table IV there is no marked difference in the response to fluids given intravenously, to a dog suffering from the effects of diphtheria toxin and a normal animal.

Table V shows a comparison of the total proteins before and after the administration of isotonic salt solution in a dog which had received diphtheria toxin, one that had the renal vessels clamped for thirty minutes, fourteen days previously and

TABLE IV

SHOWING THE EFFECT OF DIPHTHERIA TOXIN ON NORMAL BLOOD VOLUME AND ON THE BODY MECHANISM FOR CONTROLLING THE BLOOD VOLUME AFTER THE INTRAVENOUS ADMINISTRATION OF 1500 C.C. OF ISOTONIC SALT SOLUTION

DOG NO.	NORMAL BLOOD VOLUMES		1-2 WEEKS AFTER DIPHTHERIA TOXIN			FOLLOWING INJECTION OF NaCl SOLUTION		
	NO. TRIALS	BLOOD (C.C.)	NO. TRIALS	DAYS AFTER	BLOOD (C.C.)	NO. TRIALS	DAYS AFTER	BLOOD (C.C.)
1 5.7 Kilo.	2	648	2	30 37	499 405	DIED DURING FIRST INJECTION 41ST DAY		
3 20.5 Kilo.	8	2117	2	5 15	1600 1764	2	9 12	2432 1380
6 10.5 Kilo.	2	1097	1	1	946	1	4	923
7 19 Kilo.	5	1367	1	14	1298	2	18 22	1737 1092

TABLE V

SHOWING THE PERCENTAGE OF TOTAL PROTEINS BEFORE AND AFTER THE INJECTION OF ISOTONIC SALT SOLUTION

	PREFLUID VALUE	IMMEDIATELY FOLLOWING FLUID	15 MINUTES LATER	1 HOUR LATER
a*	8.7	4.0	5.3	5.4
b	6.5	3.8	4.0	4.5
c	6.4	3.1	3.8	4.6

*a—A dog, 18 days after the administration of diphtheria toxin.
b—A dog suffering renal damage.
c—A bilaterally nephrectomized animal.

a bilaterally nephrectomized animal. Comparing these results with those of Table II it will be seen that they are about the same as those shown in the normal dog.

Electrocardiograms.—Electrocardiograms were made on normal animals and then repeated following the administration, intravenously, and subcutaneously, of isotonic sodium chloride solution.

When fluid was given subcutaneously one striking result was obtained, namely, lowered voltage in all leads. It was found that this change in voltage corresponded roughly to the amount of fluid administered and that as the solution was absorbed, the height of the waves returned to their normal level.

There was no evidence of an arrhythmia, or of any disturbance of conduction or of changes in the axis deviation.

Electrocardiograms were made before, during and after the intravenous injection of 2000 c.c. of 0.9 per cent sodium chloride solution. In only one case did they show a result similar to that recounted above, with lowered voltage in all leads, and in this one case it was not marked. The rhythm, rate, and contour of the curves were unchanged.

The administration of fluid to the dogs given diphtheria toxin, resulted in no change which would distinguish the graphs from those of normal animals.

Physical Findings.—Five liters of 0.9 per cent sodium chloride solution were injected subcutaneously into a fourteen pound dog. The skin over the trunk was

TABLE VI

EFFECT OF 0.9 PER CENT SODIUM CHLORIDE, GIVEN INTRAVENOUSLY, TO A DOG SUFFERING FROM THE EFFECT OF DIPHTHERIA TOXIN

	BLOOD COUNTS	TOTAL PROTEIN	HEMATOCRIT
Prefluid	9,130,000	7.4	58
Postfluid	6,460,000	5.5	71
Per cent decrease	29	27	20

tense and would pit on pressure, but there was no edema of the extremities. When allowed to walk around the room the animal was ataxic, probably from the amount of fluid he was forced to carry. He salivated and began almost immediately to urinate. Auscultation of the heart at this time did not reveal any arrhythmia or other unusual findings. Twelve hours later he had entirely recovered, was playful and apparently had suffered no bad effects from the experience.

Sodium chloride solution was administered intravenously to normal dogs in amounts up to 2000 c.c., fifteen to twenty minutes being taken for the injection.

Following this procedure there was no edema to be noted. There was no evidence of fluid in the chest, the lungs were clear, and there were no râles or adventitious sounds. The heart rate was slightly accelerated and the rhythm was normal.

Pathologic Changes.—Gross: Postmortem examination made on the dogs which had received diphtheria toxin showed nothing of unusual importance. The heart appeared somewhat decreased in size but the muscle did not show any gross changes nor did the heart valves. The lungs were not changed except in one case in which pneumonia was present. In the dogs that had had the renal vessels clamped

there were numerous adhesions to surrounding tissues but the kidneys were grossly unchanged. No enlargement of the spleen was noted in any dog coming to autopsy following the administration of fluid.

Significant findings in the dogs suffering from the effect of toxin, into which fluid had been injected were surprisingly lacking. There was some evidence of increased free fluid in the body cavities but except for one dog that contracted pneumonia there were no changes in the lungs. This was also the case in the dog whose kidney vessels had been clamped off bilaterally and in a dog whose kidneys had been removed two days previously. The intestines were usually filled with fluid and the abdominal muscles in the dogs that had had diphtheria toxin were soft and flabby both in those which had and had not received isotonic salt solution intravenously.

DISCUSSION

The original aim of this study was that some light might be thrown upon the safety of administering large quantities of fluid and the hope that some relatively simple index might be found that could be used as a warning before the margin of safety was passed.

Large amounts of fluid were given to normal and pathologic animals and no demonstrably harmful effects were seen. There was no increase in blood volume, as measured by the dye method, no persistent change in the urinary output and no changes in the electrocardiograms, which might be interpreted as indicating danger. Because there were no evident effects, no index was found that would indicate the amount of fluid that could be safely administered. However, blood dilution did occur on the basis of red cell counts and hematocrit and blood protein determinations, which had no detectible effect on the normal animal.

The amount of fluid given was proportionately far in excess of that amount usually given patients, and the time of administration was much more rapid that that usually employed with human beings, whether given subcutaneously or intravenously. The fluid administered intravenously was not given over a period of several days, which might have caused a breakdown in elimination. However, there was no evidence of any such breakdown in dogs given three liters of isotonic salt solution subcutaneously, on three successive days.

The only unusual result obtained in the study of the electrocardiographic tracings, taken before and after the administration of fluid, was that the voltage was decreased by the introduction of 0.9 per cent sodium chloride solution subcutaneously. This fact should be of clinical importance in cases where edema of varying grades is presented and where lowered amplitude of the waves is taken to indicate myocardial weakness.

In the dogs given diphtheria toxin the results did not entirely correspond to those reported by Stewart.[12] This author interpreted a progressive lowering of the voltage of the electrocardiogram as indicating myocardial damage. The dogs of our series showed lowering of voltage as their condition became worse but in two instances this voltage returned to normal, in one of these the day before the dog died.

The urinary changes were not very significant. They showed that the urinary output alone could not account for the rapid elimination of the fluid from the cir-

culating blood following the intravenous administration of fluids and also that the lowering of serum proteins in the blood was not accounted for by the elimination of protein in the urine. No evidence of permanent damage to the concentrating power of the kidney was shown.

The index of tissue thirst as determined by the McClure-Aldrich skin test was totally unreliable in a large series of dogs.

Although the fluid injected was usually at body temperature, the few times when it was below this level, produced no ill effect except coarse tremors when given intravenously. The speed of injection seemed to cause no difficulty and although no blood pressure tracings were taken, any shock which might have been produced by as much as 100 c.c. per minute for fifteen to twenty minutes, caused no apparent severe physical reaction, nor were there any deaths which might be attributed to this cause.

It was noted that accompanying the increase in urinary output there would be an increase in salivation and at times the dog would vomit fluid. At postmortem there was a large amount of fluid in the gastrointestinal tract. It would seem that this avenue of escape would have an important bearing on postoperative dilatation of the stomach where large quantities of fluid are given.

The studies of blood volume as determined by the dye method occupied a major interest in the experiment. The value for individual blood volumes being relatively constant, it was thought that if any changes could be shown to occur following the administration of fluid, that this change could be used as an index. Since taking a presumed normal from an average figure, found by other investigators, did not give a reliable value for an individual animal and because different animals of the same weight vary as to blood volume, it was thought that the best way to establish a normal was to determine repeated volumes on the same dog. The average of these was then taken as a normal, providing the variance was close to 100 c.c. Usually the variation was not this great. The number of determinations varied from three to ten or more.

An attempt was made to determine repeated blood volumes on the same day according to the method of Smith,[14] but we were unable to get reliable results.

After a normal had been established, 0.9 per cent sodium chloride solution was injected and blood volume determined immediately. As can be seen by the preceding tables, there was no appreciable change in the blood volume as determined by the dye method, either on normal dogs or on those animals suffering from cardiac or kidney damage. It will be seen from Table III that a simultaneous check was made on blood dilution as indicated by the blood counts, hemoglobin, and hematocrit determinations, and the total protein content. These values indicate, without question, that there was a definite dilution of the blood following the administration of fluid. This was at total variance with the blood volume values given by the dye method.

It is almost impossible to explain the dilution of the blood, by storage of the red cells in the spleen, because the spleens seen at autopsy were of normal size. It seems unlikely that there would be such a vast storage in the liver, although this is a possibility.

It was also shown that the blood volumes determined by the dye method and perfusion in the dog, which had received no fluid, checked very closely. There was

not a similiar check, however, in the blood volumes determined by dye and perfusion, when an animal had been given fluid intravenously. Here the perfusion method showed a dilution not shown by the dye method. This fact is difficult to explain. One possibility is that the dye method measured only the circulating volume, and that with the addition of large quantities of fluid this circulating volume is kept at a constant level by the shunting of blood into capillary beds which at the time are temporarily closed off from the active circulation, so that they do not form a part of the circulating blood volume. No proof of this hypothesis is offered.

Another possibility is that more of the dye passes out into the tissues or is fixed by the reticuloendothelial cells under normal conditions than when fluid is administered.

CONCLUSIONS

1. No harmful results were demonstrated when large quantities of 0.9 per cent sodium chloride solution were given either subcutaneously or intravenously to normal or pathologic animals.

2. Electrocardiograms did not show any characteristic changes when large quantities of fluids were given intravenously either in normal animals or in those presumably suffering from myocardial damage.

3. Isotonic salt solution when injected subcutaneously, decreases the amplitude of the "R" wave in the electrocardiogram.

4. In normal animals exsanguination and perfusion give blood volumes which correspond very closely to those given by the dye method.

5. There is an increased concentration of the blood in animals suffering from the effect of diphtheria toxin.

6. The blood volumes as determined by the dye method show no increase after the intravenous injection of large amounts of fluid. This does not agree with the amount indicated by the dilution of the blood as shown by repeated determinations of the erythrocytes, hemoglobin, serum proteins and plasma increase.

7. No index was found which might be used as a warning against over hydration of tissue.

8. It is believed that the hematocrit is the most simple test for blood dilution following the administration of fluids postoperatively. Its use would be limited, obviously to those cases not manifesting hemorrhage or evidence of rapid hemolysis.

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LABORATORY METHODS

THE PREPARATION OF COLLOIDAL GOLD SOLUTION*

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THE preparation of colloidal gold solution is, no doubt, one of the most disappointing, time consuming and uncertain procedures encountered in routine laboratory work. Difficulties are numerous as probably has been observed by every laboratory man.

Persistent endeavor with a repeated check up on all known formulas for the preparation of colloidal gold solutions seems to have solved the problem for us.

We have found that the chief trouble was obtaining the proper, deep red and stable color, most solutions turning out purple. This was overcome by adjusting the P_H value of our distilled water. Also the control tube would generally not be decolorized in one hour using 1.7 c.c. of a 1 per cent salt solution. In adjusting colloidal gold solutions one must take into account that the sensitiveness of these solutions depends on two factors, namely the size of the colloidal particles and the P_H value of the gold solution, and that this sensitiveness must be maintained.

The method we have adopted for the preparation of colloidal gold solutions is simple, time saving and most satisfactory as tests on known positive as well as negative spinal fluids have shown this solution to be not only reliable but also fairly stable, more so than any other solution purchased previously from Laboratory Supply Houses.

METHOD OF PREPARATION

To 500 c.c. freshly distilled water (Precision Still) with a P_H of 6.8-7.0 add 5 c.c. of an aq. 1 per cent gold chloride solution and 25 c.c. of freshly prepared 0.5 per cent potassium carbonate sol (Merck's Blue Label).

Bring to boil, remove from flame and then add while shaking vigorously a few drops of Merck's Neutral Formaldehyde, diluted to about 10 per cent with distilled water, drop by drop. Three to five small drops are usually sufficient. A deep red color develops almost immediately. Cool and let stand tightly corked until next day when it is ready for titration.

TITRATION OF COLLOIDAL GOLD SOLUTION

Set up 11 Wassermann tubes and place in each tube 1.7 c.c. of salt solution of increasing strength, ranging from 1.0 per cent to 2.0 per cent. For instance: the first tube receives 1.7 c.c. of a 1.0 per cent salt solution, the second 1.7 c.c. of a 1.1 per cent salt solution, the third tube 1.7 c.c. of a 1.2 per cent salt solution, and so on until the eleventh tube is reached which receives 1.7 c.c. of a 2.0 per cent salt

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solution. The various salt solutions can be prepared from a 2.0 per cent solution as follows:

Tube No. 1 1.0 c.c. 2.0% salt sol. and 1.0 c.c. H_2O
Tube No. 2 1.1 c.c. 2.0% salt sol. and 0.9 c.c. H_2O
Tube No. 3 1.2 c.c. 2.0% salt sol. and 0.8 c.c. H_2O
Tube No. 4 1.3 c.c. 2.0% salt sol. and 0.7 c.c. H_2O
Tube No. 5 1.4 c.c. 2.0% salt sol. and 0.6 c.c. H_2O
Tube No. 6 1.5 c.c. 2.0% salt sol. and 0.5 c.c. H_2O
Tube No. 7 1.6 c.c. 2.0% salt sol. and 0.4 c.c. H_2O
Tube No. 8 1.7 c.c. 2.0% salt sol. and 0.3 c.c. H_2O
Tube No. 9 1.8 c.c. 2.0% salt sol. and 0.2 c.c. H_2O
Tube No. 10 1.9 c.c. 2.0% salt sol. and 0.1 c.c. H_2O
Tube No. 11 2.0 c.c. 2.0% salt sol. and 0.0 c.c. H_2O

Each tube now contains 2.0 c.c. of a salt solution of the various percentages from 1.0 to 2.0 per cent. Mix well and discard 0.3 c.c. from each tube, thus leaving 1.7 c.c. Now add to each tube 5 c.c. of the freshly prepared colloidal gold solution. The tube, containing the lowest percentage of salt and showing complete decolorization after one hour, indicates the strength of the salt solution to be used. For instance: if decolorization of 5 c.c. colloidal gold solution occurs in the tubes containing the higher percentage of salt solution down to and through 1.2 per cent in one hour, but no decolorization below this percentage, then the 1.2 per cent salt solution is the solution to be used in the control (twelfth) tube and 0.4 of this strength or 0.48 per cent salt solution in the other 11 tubes.

TABLE I

SHOWING RESULTS OF 107 SPINAL FLUID EXAMINATIONS

NO. OF CASES	DIAGNOSIS	CELL COUNT	GOLD CURVE	WASS.	BLOOD WASS.	BLOOD KAHN
65	Normal	3-5	Normal	Neg.	Neg.	Neg.
14	Syphilis	Increased	Syphilitic	4-plus	4-plus	4-plus
1	Syphilis	Increased	Syphilitic	3-plus	4-plus	4-plus
1	Syphilis	Increased	Syphilitic	Anticompl.	4-plus	4-plus
1	Syphilis	3	Syphilitic	Neg.	4-plus	3-plus
3	Paresis	Increased	Paretic	4-plus	4-plus	4-plus
1	Encephalitis	8	Syphilitic	Neg.	Neg.	Neg.
1	Encephalitis	5	Syphilitic	Neg.	Neg.	Neg.
1	Encephalitis	12	Syphilitic	Neg.	Neg.	Neg.
1	Encephalitis	75	Syphilitic	Neg.	Neg.	Neg.
1	Encephalo-Meningitis	1288	Center-Curve	Neg.		
1	Brain Tumor	58-3050	Normal	Neg.	Neg.	Neg.
4	Meningitis	Increased	Meningitic	Bact. Exam.: meningococci		
4	Meningitis	Increased	Meningitic	Bact. Exam.: pneumococci		
3	Meningitis	Increased	Meningitic	Bact. Exam.: streptococci		
5	Tubercle Meningitis	Increased	Meningitic	Guinea Pig Inoculation: Positive t.b.		

It will be seen from Table I of cases that 15 definitely syphilitic cases with strongly positive Wassermanns on both spinal fluid and blood, also with positive blood Kahn reactions, gave typically syphilitic type curves with the colloidal gold reaction. Two other syphilitic cases, one with a negative and the other with an anticomplementary reaction in the spinal fluid Wassermann tests, but with strongly positive blood Wassermanns, gave typical luetic curves with the colloidal gold.

Three cases, diagnosed as paresis, with strongly positive spinal fluid and blood Wassermanns and positive blood Kahn reactions, all gave definitely positive paretic curves with the colloidal gold.

Four cases of encephalitis with negative spinal fluid and blood Wassermanns and Kahn reactions gave syphilitic curves with the colloidal gold.

It has not been unusual in our experience to obtain typically syphilitic type curves in cases clinically diagnosed as encephalitis, with the spinal fluid and blood Wassermanns negative.

One case, diagnosed as encephalomeningitis, and with a high cell count gave a center curve, a little to the right of a typical syphilitic type curve and extending into the meningitic curve region.

One case with a brain tumor with negative spinal fluid and blood Wassermanns gave no alteration from the normal with the exception of a high and varying cell count.

Sixteen cases, clinically diagnosed as meningitis and bacteriologically proved as such, all gave typical meningitic type curves.

The other cases reported checked with the negative clinical diagnosis of syphilis and the negative spinal fluid and blood Wassermanns as well as Kahn reactions and gave normal colloidal gold curves.

Such findings are not possible unless the point of decolorization and sensitiveness of the colloidal gold solution is accurately balanced. This balance is essential and accurate titration seems to be indicated, no matter what method is used in the preparation of the colloidal gold solution.

The method herewith submitted for the preparation of this solution is not original with us but was adopted as the best from our experience.

Titration of the colloidal gold solution, to determine the correct percentage of salt solution to be used, will help solve the difficulty that many laboratories have had and are having in its preparation.

THE PHARMACOLOGIC STANDARDIZATION OF PARATHORMONE*

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THE assay of parathormone (parathyroid extract Collip), or other parathyroid extracts, due to the peculiar criteria required to measure their potency, is comparatively difficult. Collip and other investigators[1] have shown that the active principle of this material quantitatively affects the mechanism controlling the calcium and phosphorous concentration within the blood stream. The first of these physiologic processes to be employed, and the one which is now believed to be the most reliable for the purpose of measuring the degree of activity of parathyroid extract, is concerned with blood serum calcium. Collip, Clark, and Scott,[2] using a modified Kramer-Tisdall method,[3] determined the serum calcium values in parathyroidectomized dogs. By means of subcutaneous injections of parathyroid

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extract, they were able to maintain a calcium level which prevented the death of these animals. Their next step was an attempt to influence the serum calcium of normal animals. They were able to do this quantitatively on healthy, untreated dogs.

Test Subjects.—In selecting experimental animals for the routine testing of parathormone, the requirements of the chemical test to be used must be kept in mind. The collected blood must not be contaminated by extraneous calcium. Care must be taken, therefore, that it does not touch the hair or skin of the subject or the hands or clothing of the operator. This requirement eliminates the possibility of collecting blood from an incised vein in the cat, rabbit, guinea pig, rat, or mouse. Cardiac puncture, likewise, is impractical since the procedure, always dangerous, causes frequent deaths and consequent changes in the identity of the test group. Collip[4] found that the response of normal rabbits to parathyroid extract, even in massive doses, was not uniform.

On the other hand, it was found that healthy dogs maintain a fairly constant normal calcium level, are relatively long lived, and can be obtained of such a size that the withdrawal of 20 c.c. of blood would not appreciably affect the total blood volume over a twenty-four-hour test period.

Selection of Dogs.—Parathormone is evaluated by the degree to which it is capable of producing a rise in the blood serum calcium of normal dogs. The calcium response of each individual, therefore, must be fairly uniform. In selecting the members of a group for this purpose, it is necessary to exclude all those which might be expected to show gross inconsistencies at any time. This classification includes all females, since a variable response is usually obtained during the estral cycle; old males, because senility reduces tolerance; very young males, because the calcium picture during rapid growth usually shows great variations; and males with respiratory, intestinal, or cutaneous diseases. Or, to state the case positively, a good test dog for parathormone should have the following qualifications:

Sex	Male (mature)
Weight	15-25 kg.
Health	Free from gross evidence of disease

It is our experience that, from 100 dogs collected indiscriminately, approximately 20 are suitable for parathormone testing.

The Individual.—Parathormone unitage is based directly upon the milligram rise of calcium per 100 c.c. of blood serum. Therefore, the individual when injected with a given number of units of the substance should show an increase in blood calcium, commensurate with the size of the dose.

One unit of parathormone is defined as one one-hundredth of the amount required to cause a rise of 5 mg. of calcium per 100 c.c. of blood in a 20 kg. dog. It is obvious that one unit would produce a change too small to be of any value and at the other extreme, 100 units may produce results which are injurious to the health of the test animal. It has been found that a dose of 66 units causes no injury and gives the most consistent results. If 20-unit material is used, this dose is 3.3 c.c. per 20 kg. dog and should be responsible for a blood calcium rise equal to 0.66 of 5 mg. or 3.3 mg. per 100 c.c. of blood serum.

This is the standard or theoretical normal rise for the individual. All dogs

do not respond alike, but they should be consistent from week to week and show a rise equal to the normal plus or minus 2. An increase of less than 1 mg. of calcium per 100 c.c. of blood serum should not be considered.

Occasionally one member of a group will show no rise after the customary dose of parathormone. This individual must be replaced in the group by one whose response has proved to be fairly constant. The dog failing to show the specified calcium rise must not be used for testing until his unusual behavior has been corrected. It is often the less expensive course to discard such an animal immediately.

The Group.—A parathormone test group consists of not less than 5 dogs, but may be made up of as many more as the amount of material to be tested, the cost of the animals, and the time available will permit.

The extract in doses sufficient to cause a rise of 3.3 mg. of calcium per 100 c.c. of blood serum is responsible in some cases for a long continued hypercalcemia. It is advisable, on this account, to use a group but once each week, and it is preferable to use it not more often than once every two weeks.

When a group has been standardized by the injection of a stock preparation of known value, a certain amount of regularity must be maintained in the matter of calcium stimulation to get the most consistent results. The animals should receive a dose of parathormone, whether it be test material or stock, at their regular injection time. The amount of this dose must be sufficient to give 3.3 mg. of calcium rise per 100 c.c. of blood serum, or a dose of 3.3 c.c. of 20-unit parathormone per 20 kg. of dog weight.

Diet and Environment.—A diet, constant in composition and in amount per dog, seems to be essential. Any marked change in either of these factors affects the calcium which is available to parathormone influence, even though the normal remains the same. A prepared diet, containing dried meat, cod liver oil, molasses, dried buttermilk, wheat germ meal, wheat bran, corn cereal, oat cereal, barley malt, and salt, is used in our kennels with complete satisfaction. An occasional raw bone and fresh vegetables may be added to keep the dog's appetite healthy.

A minimum of exercise, and habituation to new living conditions are to be kept in mind, also, when using new dogs.

Bleeding and Injection.—Blood is usually collected early in the morning of the day on which the dogs receive an injection of the extract. Their feeding is omitted after this bleeding until the final collection has been made on the following morning.

Apparatus: Glass syringes, 10 c.c. capacity
1.25 in., 19 guage hypodermic needles
Glass centrifuge tubes, 15 c.c. capacity, ungraduated
Rubber stoppers to fit centrifuge tubes

The dog to be bled is placed on a table and held on his side by two assistants. No other means for holding a dog quietly and securely has been found satisfactory. The hair is clipped for a distance of 4 to 6 inches from the ankle upward on the lateral surface of either hind leg. Tourniquet-like pressure, applied at the knee by one of the assistants, will cause the saphenous vein to stand out prominently. This vein will be found to run in a diagonal course from the posterolateral aspect of the knee down to and across or around the ankle. With pressure on the vein

maintained, blood is withdrawn by inserting the needle (fitted to a syringe) through the skin directly into the lumen of the vein. A gentle traction on the plunger of the syringe permits the barrel to fill. To avoid a hematoma, light digital pressure must be applied at the point of entry of the needle as it is being removed. Much more pressure is added after the needle is out, and the grip above the knee is loosened at the same time. Under these conditions, the minute wound seals itself very quickly. The blood is gently expelled from the syringe, immediately, into a calcium-free glass centrifuge tube, and the blood-stained syringe is placed in distilled water to prevent sticking.

In the dog, the peak of the blood calcium rise occurs from sixteen to eighteen hours after subcutaneous injection of the parathyroid extract. Therefore, the animals must be injected so that the final bleeding can be taken care of conveniently at the proper time, that is, during working hours. It has been customary, here, to make the parathormone injections between 4 and 5 o'clock in the afternoon of the day on which the normal blood was taken, and the final bleeding on the following morning between 8 and 9 o'clock.

All administrations of parathormone are made subcutaneously on the side of the dog. These injections are placed well toward the ventral surface in the loose tissue found there. The skin at the point of entry of the needle is pinched between the thumb and forefinger, just as the needle is withdrawn, to prevent leakage, and the injected area is massaged thoroughly to insure dispersion of the dose.

Glassware.—All the glassware used in the test must be calcium-free. It is allowed to stand in hot cleaning fluid (100 gm. of potassium dichromate to 4 liters of commercial sulphuric acid) for one hour; then passed in succession through 7 changes of distilled water. For the purpose of drying, it is placed in an inverted position on filter paper for about three hours. The drying process is completed in an electric oven at 100 to 110° C.

The steel needles, graduated syringes, and rubber stoppers are not placed in the acid bath but are thoroughly brushed and cleaned in distilled water, and then, with the exception of the stoppers, are taken through the same procedure as the rest of the glassware. The stoppers are dried at room temperature on filter paper.

Calcium Determination.—The Tisdall modification of the Kramer-Tisdall method[5] is employed for the quantitative determination of the blood serum calcium. Clean glassware, and practice and care in technic are the chief requirements for making this an accurate test. For the practical purpose of standardizing parathormone, this method suffices.

The gasometric calcium method of Van Slyke and Sendroy[6] and the colorimetric method of Roe and Kahn[7] may be more precise, but they are much more complex in manipulation.

Calculation of Unitage.—Since the parathormone unit is defined as one one-hundredth of the amount required to raise the calcium level of a 20 kg. dog, 5 mg. per 100 c.c. of blood serum, the unitage calculation takes the following form:

$$\frac{\text{Group Av. Ca. rise (mg. per 100 c.c.)}}{\text{Parathormone dose (c.c. per 20 kg.)}} = \text{mg. Ca. rise per c.c. parathormone}$$

One unit is responsible for 0.05 mg. calcium rise per 100 c.c. of blood serum.

$$\text{Then the } \frac{\text{mg. Ca. rise per c.c. parathormone}}{\text{0.05 mg.}} = \text{units per c.c. parathormone}$$

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A NOTE ON THE LIEBERMANN-BURCHARD COLOR REACTION FOR CHOLESTEROL*†

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IT IS now a common observation that the Liebermann-Burchard reaction for cholesterol under uncontrolled conditions is not a reliable one. Temperature, intensity of light and time are all factors which tend to affect this reaction (Myers and Wardell,[1] Cornell,[2] Elman and Taussig,[3] Mattice‡). Consequently, we are frequently confronted with the difficulty of obtaining duplicate results to check a single sample of blood. The literature abounds with suggestions for eliminating such discrepancies but we have found none of these efficacious.

During the course of our preliminary study of methods for the determination of cholesterol in the blood, we experienced great difficulty in getting checks closer than 10 per cent, irrespective of the colorimetric method employed. We finally decided to use Sackett's modification of Bloor's method[4] because of its simplicity, reliability, and inexpensiveness. However, our early determinations with this method showed a discrepancy of about 10 per cent between duplicate samples. In order to find the cause of this variation, we studied every aspect of the method. The procedure consists in adding 0.2 c.c. of plasma (or whole blood) to a mixture of 9 c.c. of alcohol and 3 c.c. of ether and subsequently evaporating the alcohol-ether extract to dryness. The cholesterol in the residue is then extracted with 5 c.c. of chloroform. To this is added 0.1 c.c. of sulphuric acid and 2 c.c. of acetic anhydride and a green color results (Liebermann-Burchard reaction).

A common argument against this method is the fact that a brown discoloration is frequently produced on rapid evaporation of the alcohol-ether extract.

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**Oliver Rea Fellow in Medicine.
‡It has been observed independently by Miss M. R. Mattice in the Biochemical Laboratory of this institution that the color produced in the Liebermann-Burchard reaction may vary from a blue to a greenish yellow depending in a large measure on the temperature at which the reaction is allowed to take place.

This is eliminated completely if the evaporation is allowed to take place slowly in an incubator at 37° C. (Rabinowitch[5]).

The two chloroform extractions as suggested by Sackett are absolutely complete; further extractions of the alcohol-ether residue with chloroform are unnecessary.

We then considered the effect of temperature, and here we found the source of our discrepancies. The simple procedure of cooling the solutions after adding the sulphuric acid and the acetic anhydride in cold running tap water, as suggested by Myers and Wardell,[1] was tried on many occasions but the checks obtained were poor. It was felt that more uniform low temperatures were desirable and, indeed, necessary. In order to fix the temperature at which the color was produced at a low level and at the same time keep the solutions in darkness, we placed our extracts in a refrigerator which maintained a constant temperature of 5° C. This procedure immediately gave us more uniform results. Still better duplicate determinations were obtained when the Liebermann-Burchard reaction was allowed to take place for five minutes in a dark closet and then another ten minutes in the refrigerator. Controlled experiments with varying amounts of standard solutions showed that the maximum color is obtained under

TABLE I

TYPICAL RESULTS OBTAINED WITH THE LIEBERMANN-BURCHARD REACTION

SAMPLE		REACTION AT ROOM TEMPERATURE		SAMPLE		REACTION AT 5° C.	
		CHOLESTEROL MG. PER CENT	MEAN DIFFERENCE PER CENT			CHOLESTEROL MG. PER CENT	MEAN DIFFERENCE PER CENT
1	a	213.	10.2	6	a	231.5	0
	b	192.2			b	231.5	
2	a	187.8	13	7	a	141.0	1
	b	164.8			b	139.5	
3	a	243.5	8	8	a	161.0	0.9
	b	264.0			b	159.5	
4	a	273.7	10.4	9	a	216.7	0
	b	246.7			b	216.7	
5	a	307.5	15.2	10	a	213.0	0
	b	264.0			b	213.0	

these conditions. The solutions were removed from the refrigerator one at a time and read against the standard cholesterol solution prepared under the same conditions. When readings are made with artificial light (shades of green are better differentiated with artificial light), it is advisable not to read more than six solutions against the same standard, since heat and light alter the green to a greenish yellow fairly rapidly and thus tend to vitiate the results.

Table I indicates the typical results obtained when the color reaction occurs at room temperature and those obtained with the procedure outlined above. The simple expediency of allowing the reaction to take place at a temperature of

refrigeration (5° C.) has given us results which check within 2 per cent for a single specimen. Only rarely do we find discrepancies greater than this.

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A METHOD OF MEASURING CHOLESTEROL*

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INTRODUCTION

THE final estimation of cholesterol by the colorimetric method is difficult. This is so because the green color developed for the reading on the colorimeter is a factor which varies independently of the quantity of cholesterol. The standard if permitted to age before using in a determination will possess a green color which is different from that produced in a freshly prepared standard. The green color which develops in either the standard or the unknown cholesterol solution has only a fleeting maximum color intensity which rapidly fades. This change is due to the conversion of the green color to yellow producing many yellowish shades of green which are entirely unreliable for matching.

It was felt that possibly the yellow color could be utilized as a method of measurement of the cholesterol, in as much as such a color was very easy to match. This yellow color, it was found, could be very easily produced, if the cholesterol solutions made up as for a green comparison were permitted to remain in a dark cabinet at about 20° C. for a period of twelve to twenty-four hours. Moreover, the yellow color was found to be far more stable than the green.

METHOD

The procedure is a modified Myers-Wardell.[1] One cubic centimeter of whole blood which had been collected in a sterile oxalate tube (oxalate equivalent to 2 c.c. of 1 per cent solution) was placed in an extraction thimble in which had been placed loosely packed fat-free filter paper. This blood was dried for three hours at a temperature of 70 to 80° C. and then extracted continuously with 20 c.c. of chloroform at 70 to 80° C. for two and one-half hours. The chloroform extract was made up to 25 c.c. at 20° C. Into each of two tubes was placed 0.1 c.c. of concentrated sulphuric acid and 2 c.c. of acetic-anhydrid. Into one tube was place 5 c.c. of the

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unknown blood cholesterol extract and into the other tube 5 c.c. of a standard cholesterol solution (5 c.c. of the standard contained 0.4 mg. of cholesterol). Each tube was made up to 10 c.c. with chloroform, stoppered, and placed in a 20° C. water-bath in a dark cabinet. In fifteen minutes the tubes having developed their maximum green color, were matched, the unknown against the standard, in a Dubosque colorimeter. The tubes were immediately replaced in the 20° C. bath and returned to the dark cabinet where they remained for a period of twelve to twenty-four hours. They were then removed from the water-bath and the unknown matched against the standard. The number of milligrams of cholesterol per 100 c.c. of whole blood was found by the calculation:

$$\frac{\text{Reading of standard} \times 200}{\text{Reading of unknown}}$$

Known concentrations of cholesterol were matched against the standard. The quantity of the known cholesterol solution used was 5 c.c. The results as measured in 5 c.c. was found by the calculation:

$$\frac{\text{Reading of Standard} \times 0.4}{\text{Reading of unknown}}$$

Duplicate experiments were performed upon all specimens. The average results are presented.

RESULTS

The comparative results of both green and yellow color estimations are presented in 10 known, pure cholesterol solutions and in 42 unknown blood cholesterol extractions.

TABLE I

CHOLESTEROL IN PURE SOLUTION, MEASURED IN MG. PER 5 C.C.	CHOLESTEROL AS MEASURED IN MG. PER 5 C.C. USING GREEN COLOR	CHOLESTEROL AS MEASURED IN MG. PER 5 C.C. USING YELLOW COLOR
0.10	0.02	0.12
0.20	0.24	0.20
0.40	0.33	0.41
0.56	0.48	0.54
0.60	0.50	0.60
0.70	0.60	0.66
0.80	0.90	0.82
1.00	1.00	1.00
1.12	1.40	1.20
3.00	4.00	3.00

DISCUSSION

It is important that accurate procedures in cholesterol estimation be devised, methods that involve a rapidity which gravimetric methods do not possess. The colorimetric method offers that rapidity and in addition, a fair accuracy. Therefore any procedure which increases the accuracy of this method is justified.

Table I presents the comparative cholesterol estimations as measured by the green and yellow colors produced in cholesterol solutions of known strength. It is

TABLE II

SPECIMEN	READING OF STANDARD	READING OF UNKNOWN IN		DIFFERENCE	MG. CHOLESTEROL PER 100 C.C. BLOOD IN		DIFFERENCE
		GREEN	YELLOW		GREEN	YELLOW	
1	15	19	21	2	157	143	14
2	15	24	26	2	125	115	10
3	15	20	22	2	150	136	14
4	15	23	25	2	130	120	10
5	15	28	30	2	108	100	8
6	15	32	30	2	93	100	7
7	15	24	26	2	124	115	9
8	15	25	23	2	120	130	10
9	15	24	22	2	125	136	11
10	10	27	30	3	74	66	8
11	15	27	29	2	111	113	2
12	15	19	18	1	157	166	9
13	15	25	24	1	120	125	5
14	15	31	32	1	99	96	3
15	15	20	24	4	150	125	25
16	15	22	25	3	136	120	16
17	15	27	28	1	111	107	4
18	15	30	30	0	100	100	0
19	15	33	30	3	90	100	10
20	15	33	30	3	90	100	10
21	15	28	31	3	108	99	9
22	15	32	30	2	93	100	7
23	15	24	27	3	124	111	113
24	15	25	22	3	120	136	16
25	15	24	20	4	125	150	25
26	10	30	27	3	74	66	8
27	15	27	29	2	111	103	8
28	15	19	18	1	157	166	9
29	15	19	22	3	157	136	21
30	15	24	26	2	124	115	9
31	15	20	22	2	150	136	14
32	15	23	24	1	130	124	6
33	15	13	15	2	230	200	30
34	15	28	25	3	107	120	13
35	15	21	18	3	142	166	24
36	10	27	26	1	74	76	2
37	15	24	22	2	125	136	11
38	15	20	19	1	150	157	7
39	15	21	18	3	142	166	24
40	15	17	20	3	176	150	26
41	15	20	17	3	150	176	26
42	15	16	14	2	187	294	27

evident from this table that in pure cholesterol solutions the estimation by the use of the yellow color is much more accurate than by the use of the green color.

Table II presents the results of the cholesterol estimation of blood specimens by both the green and yellow colors. Here, too, it is evident that there is a difference in the amount of the estimated cholesterol when the two colors are used.

Inasmuch as the yellow color resulted in the more accurate cholesterol estimations in the pure cholesterol solutions and inasmuch as the cholesterol estimations in the blood specimens by the yellow color were different from those obtained by the green color in both positive and negative directions, it is felt that the measurement of cholesterol by means of the yellow color is more accurate than when the green color is used.

SUMMARY

A method of preparing cholesterol for estimation is described. This method is more satisfactory and more accurate than the original colorimetric method of estimation.

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A SIMPLE METHOD FOR THE PREPARATION OF HEMATOPORPHYRIN*†

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INTRODUCTION

THERE are a number of well characterized diseases in which the group of substances named porphyrins seems to play a definite rôle. Hematoporphyrin is a member of this group. In the last few years new light has been thrown on the clinical picture by the experimental data which has been accumulated by investigations on the physiologic, pathologic, photosensitizing, acute intoxication and chronic intoxication behavior of hematoporphyrin. Drs. Cornbleet, Bachem, and Reed of the staff of the University of Illinios College of Medicine have been studying the biologic behavior of this substance. They were handicapped because of the lack of reasonable quantities of pure hematoporphyrin.

PROBLEM

It was this difficulty which suggested a study of the available methods. They were found satisfactory for the most part but so time consuming, that for the preparation of relative large quantities of pure hematoporphyrin they were impractical. By modification and combination of some of the desirable steps in the older methods, it was possible to easily prepare relatively large quantities of pure neutral hematoporphyrin, free from protein, iron, and other undesirable by-products yet possessing all the physical and chemical, fluorescent and spectroscopic properties attributed to it.

SOURCE

The most common source of hematoporphyrin is the blood. It has been prepared from all types of blood pigments and its derivatives as hemoglobin, oxyhemoglobin, carbonmonoxide hemoglobin, reduced hemoglobin, methemoglobin, hemin, hematin, and hemachromogen.

Hematoporphyrin has been found in the contents of the stomach and intestinal tract after the introduction of acids in the stomach.

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*From the Department of Physiological Chemistry, College of Medicine of the University of Illinois.

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Garrod in 1892-1900[1] found hematoporphyrin in the urines of normal and pathologic patients afflicted with cirrhosis of the liver and obstipation.

MacMunn in 1885[2] and Hammarsten in 1891[3] found hematoporphyrin in urines from patients who were poisoned with sulphonal and trional.

H. Fischer and Kogl in 1923[4] prepared hematoporphyrin from highly colored feathers.

H. Fischer and Zeile in 1929[5] synthesized hematoporphyrin in small quantities.

METHOD OF PREPARATION

In this method the source of hematoporphyrin was oxalated blood. Equal parts of oxalated blood and concentrated sulphuric acid were stirred until well mixed, and then allowed to cool. Four volumes of distilled water were added to one volume of this solution, which was then further allowed to cool. The solution was then filtered. The filtrate, a clear red solution, was neutralized with concentrated sodium hydroxide solution. Hematoporphyrin in the form of a brown amorphous precipitate appeared at or slightly to the acid side of the neutral point. The mixture was then filtered. The precipitate and filter paper were dissolved in just a sufficient amount of concentrated sulphuric acid and cooled. This acid solution was diluted with distilled water as above, one part solution to four water, further allowed to cool, and then neutralized as above with concentrated sodium hydroxide solution. Again the same brownish precipitate appeared. The mixture was then tested for the presence of protein by the Biuret test on a small portion of the filtrate. A positive test indicated the necessity of a repetition of the solution and precipitation process. The process was repeated as often as necessary to free the solution from protein. When the mixture was protein free, the precipitate from the last neutralization was allowed to settle out. The supernatant fluid was removed and replaced with distilled water. The mixture was then dialyzed in a collodion bag against distilled water until sulphate and iron free, and dried at room temperature.

RESULTS

a. Physical Properties.—Hematoporphyrin prepared by this method was in precipitate form, dark brown, flaky, and amorphous. When dry has a dark red, almost black, color, with a definite violet shimmer and is friable.

b. Chemical Properties.—The product was easily soluble in concentrated sulphuric acid, hydrochloric acid, nitric acid, potassium, sodium, ammonium hydroxide, dilute acetic acid and sodium carbonate: slightly soluble in dilute sulphuric acid, glacial acetic acid, methyl alcohol, benzol, nitrobenzol, petroleum ether, acidified ethyl alcohol: insoluble in water, 96 per cent ethyl alcohol, ether, chloroform. In acid or alkaline solution it can be precipitated at neutrality point with neutral salts as magnesium sulphate, ammonium sulphate, and potassium chloride. A test for iron was negative. At 100° C. it is destroyed giving a pyrrol odor.

c. Fluorescence.—When ultra-violet light is passed through a chamber containing a suspension in distilled water of this preparation it gives a brown fluorescence, in acid solution a dark red, and in alkaline solution a purple red.

d. Spectrum Absorption.—The material was examined spectroscopically with a Hiliger Spectroscope. The solutions contained 0.05 per cent of hematoporphyrin,

and the acid used was sulphuric acid and the alkali, sodium hydroxide. The bands obtained were found similar to those attributed to acid and alkaline hematoporphyrin. No evidence has been found in the literature for neutral absorption bands.

TABLE I

	Alkaline			
	I	II	III	IV
½ cm.	620 uu—weak	566 uu—weak	539 uu—weak	517 uu—strong
1 cm.	615 uu	576 uu	540 uu	519 uu

	Acid		
	I	III	
½ cm.	594 uu—narrow	550 uu—broad	Absorption
1 cm.	594 uu	(570) 550 uu	Beginning at 490 uu

	Neutral	
	I	II
½ cm.	536 uu—very broad	500-510 uu
1 cm.	566 uu	510 uu

DISCUSSION

The method described is a composite of the desirable features from the following methods.

Laidlaw[6] was among the first to use whole blood and concentrated sulphuric acid. The resulting mixture was allowed to stand from three to four days, then extracted with alcohol and evaporated to dryness.

Hoppe-Seyler[7] was the earliest to describe a method and he named the material hematoporphyrin. His method uses hemin which necessitated the production of hemin.

Eschbaum[8] modified the Hoppe-Seyler method in that he used whole blood and concentrated sulphuric acid. The resulting mass was extracted with absolute alcohol and neutralized with an alcoholic solution of sodium hydroxide.

The Nencki-Sieber method[9] is by far the most elaborate. Their method as modified by Willstatter and Fischer[10] is as follows: Hemin is treated with a solution of hydrogen bromide in glacial acetic acid, then allowed to stand for five days. The solution is then diluted with water, filtered, and neutralized with sodium hydroxide. The product is freed of impurities by washing the precipitate with a solution of dilute acetic acid until free of chloride. Dhere-Sobolewski[11] added dialysis of the solution after precipitation with sodium hydroxide.

The methods mentioned are all time-consuming and involve many technical procedures. With the exception of the Nencki-Sieber method the product obtained is not a pure hematoporphyrin. All give a poor yield.

SUMMARY

The method described is simple and rapid because it is applied to whole blood. The quantity of concentrated sulphuric acid is important since higher concentrations of acid will tend to form compounds other than hematoporphyrin. Lower concentrations tend to allow precipitation of the pigment. This method completely removes the protein. Dialysis is of very definite value because the iron salts and sodium sulphate dialyze through while pigment remains in the bag. Finally the product is a pure neutral hematoporphyrin.

CONCLUSIONS

1. A simple method for the preparation of hematoporphyrin is described.
2. The product obtained by the method is a neutral hematoporphyrin.
3. The properties of the product are those of hematoporphyrin.
4. It is free from iron, the sulphate ion and protein.

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A TURTLE BOARD WHICH SERVES SEVERAL LABORATORY PURPOSES*

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THE turtle board described below, devised in the Laboratory of Pharmacology of the George Washington University School of Medicine, has been found to be a valuable addition to the locker list and a description was thought advisable.

The board consists of a base (*A*) and two uprights (*B1, B2*), and closely resembles the inverted form of the ordinary type of operating board used for mammals. The base (*A*) and uprights (*B1, B2*) are made of dressed white pine, six-eighths of an inch in thickness. The base (*A*) is seven and one-half inches wide and twelve inches long. The uprights (*B1, B2*) are seven inches long and one and six-eighths inches in height, and are fastened to the base (*A*) by a copper screw in the center and nails near the ends. The most satisfactory positions of

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the uprights, leg and head fasteners and holes for the head cord are shown in Fig. 1.

When the turtle is used, the animal is placed in an inverted position in the uprights (*B1, B2*) with its head toward the end of the base (*A*); the legs are

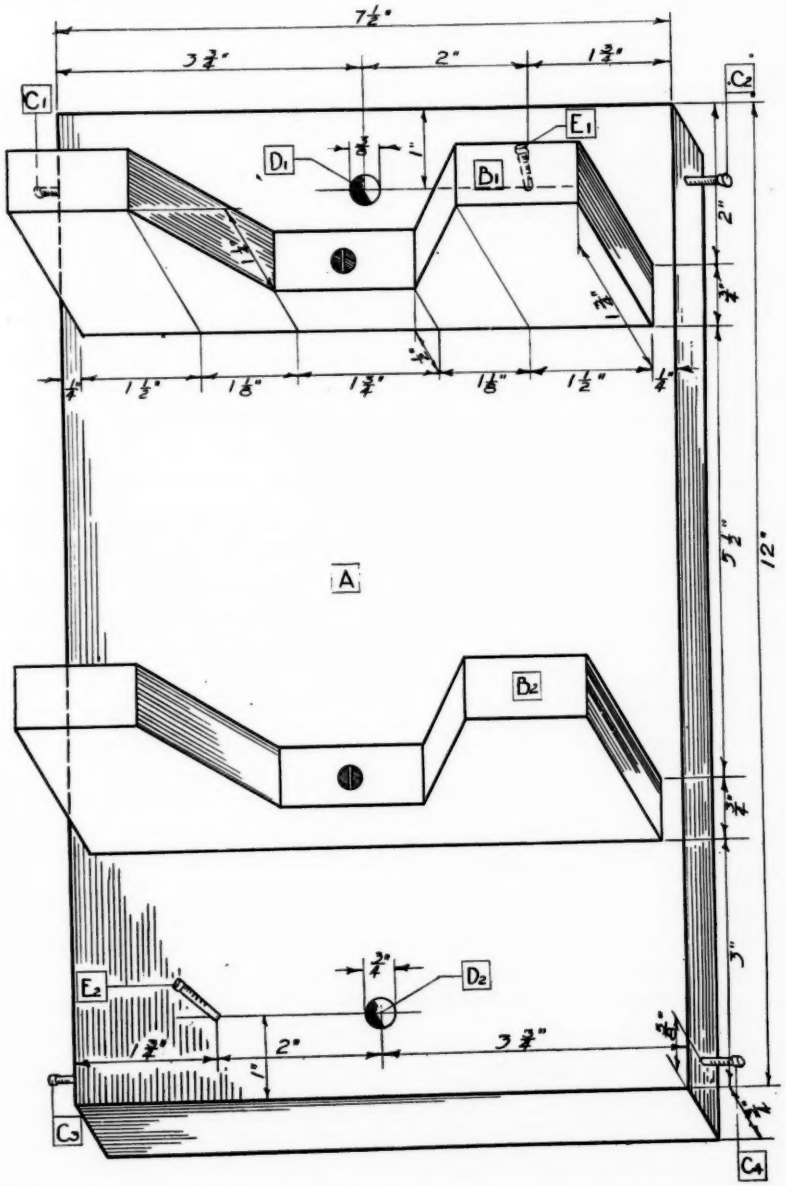

Fig. 1.—Drawn to scale. *A*, base. *B1, B2*, uprights. *C1, C2, C3, C4*, leg fasteners. *D1, D2*, holes for head cords. *E1, E2*, head fasteners.

firmly tied to the leg fasteners (*C1, C2, C3, C4*), a heavy cord is tied around the neck with the knot at the base of the head, and the free end of the cord is carried over and under the end of the base, up through the hole (*D1* or *D2*), pulled taut, and tied to the fastener (*E1*, or *E2*).

This board is adaptable without change for all the usual sizes of turtles used in the laboratory. Its small size avoids interference with stands for holding heart levers, etc., and its relatively light weight does not preclude its use for perfusion work, when clamped to an upright stand. The board, when resting on the uprights, may be used as a kymograph stand, and also as a frog board, if holes for leg fasteners are drilled in the base.

A DIFFERENTIAL STAIN FOR DRIED, UNFIXED VAGINAL SMEARS*

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IN STUDYING the action of various hormones on the genitourinary organs, the white rat has been used. During these studies vaginal smears were made daily, for consecutive weeks, on many animals. Five smears were made on one microscopic slide in order that a complete estrus cycle might be studied on a single mount. The smears were made in half-inch areas, leaving ample room at one end for labeling.

Since it was impractical to stain daily the fresh smears as they were added to the slide, it was necessary to use a stain which would give good results with dried, unfixed smears. It was found that various dyes stained well, but that the cells could not be differentiated in smears made during the characteristic phases of the reproductive cycle. Hence, it became necessary to employ a stain which would react with old and unfixed material, and also differentiate the various kinds of cells found in the smears.

The stain which finally was found to meet all requirements was Harris' hematoxylin followed later by a counterstain consisting of indigo-carmine and yellow eosin. The counterstain was made from indigo-carmine (Grubler), 0.25 gm.; eosin Y (Coleman and Bell), 1 gm.; and distilled water, 99.00 c.c. A small lump of thymol was added to prevent the growth of mold. No fixing agent was required before staining.

The staining procedure was as follows: The slides were (1) immersed in alcohol (95 per cent), for three minutes; then (2) in distilled water for one minute; (3) immersed in Harris' hematoxylin (or Delafield's hematoxylin) for twenty minutes; (4) washed in tap water for two minutes; then (5) in alkaline alcohol (70 per cent alcohol made by using a 4 per cent solution of aqua ammonia instead of distilled water); (6) soaked in the counterstain (indigo-carmine and eosin), eight hours or overnight; (7) washed quickly in tap water; (8) dehydrated quickly in absolute alcohol; then (9) cleared in xylol; and finally (10) mounted in balsam.

In the application of this technic, the cells could be identified as follows: The nuclei of the epithelial cells were gray, while the cytoplasm was lavender. The cornified cells were bright pink. The leucocytes had dark nuclei while the cytoplasm was clear. The red blood cells were pink.

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Vaginal smears made in this way may be handled the same as paraffin sections in regard to destaining after the hematoxylin. No destaining is required, however. In fact, it was found that the stain was intensified both by the tap water used to wash off the hematoxylin and by alkaline alcohol.

This process of staining vaginal smears requires considerable time, which, however, the excellent results in differentiation would seem to justify. It is possible to hasten the procedure somewhat by warming the staining solutions, but this is not to be recommended, because when this is done the slides are likely to become overstained.

CASE OF DYSENTERY (FLEXNER) TREATED BY BACTERIOPHAGE*

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ONE of us (S. W. J.) obtained from the Bacteriological Laboratory of Columbia University, New York Post-Graduate School, an agar slant culture of Bacilli dysenteriae (Flexner) and an effective bacteriophage, for experimental work. The Bacilli dysenteriae had been isolated from a case of acute dysentery at the Post-Graduate Hospital. The bacteriophage had been isolated from sewage, and produced a complete lysis of the organisms in four hours. Through careless manipulation S. W. J. became infected and developed an acute dysentery.

Following is the history: On Friday night November 6, 1931, three days after obtaining the culture, marked restlessness and abdominal distress developed. Several chills and an attack of vomiting followed. A large stool was then evacuated, with severe griping. Three additional watery stools were evacuated by morning. Temperature 101° F., pulse 90.

A suspicion of accidental infection by Bacilli dysenteriae (Flexner), prompted a stool examination and preparation of a bacteriophage. Five cubic centimeters of fresh bacteriophage which caused complete lysis in vitro of the organisms, in four hours was taken immediately, by mouth.

Dr. Davenport was then called to attend the case. Following are his findings:

Young man, aged thirty-five years, weight 145 pounds, appears acutely ill, temperature 101° F., pulse 90. Chest and heart normal. Abdomen is slightly distended, with generalized tenderness. Urine examination is normal. The patient was put to bed. 8 P.M.: Temperature was 102° F., pulse 94. Twelve watery stools, no blood. Slight dehydration.

Sunday 8 A.M.: Temperature 101° F., pulse 90. Marked prostration. Ten stools evacuated during night, dark brown in color, moderate amount of mucus.

Five cubic centimeters of bacteriophage, in water, given by mouth. Buttermilk and tea given ad lib.

12 noon: Temperature 99° F., pulse 80; 5 c.c. of bacteriophage in a little water, given by mouth.

8 P.M.: Temperature 99° F., pulse 80. Patient comfortable. Three stools during day. Patient sleeping at intervals.

Monday: Report of stool examination shows Bacilli dysenteriae (Flexner) present. Specific bacteriophage absent from stool.

Patient had six stools in past twenty-four hours. Large amount of mucus, no free blood. Marked prostration is present. Temperature 98° F., pulse 80.

*Received for publication, December 7, 1931.

Tuesday: Patient up and around, but is still weak. Stool examination was negative for dysentery bacilli. Positive for bacteriophage.

Complete lysis of organisms in four hours. Urine examination is normal. Weight 134 pounds.

Friday: Stool examination was negative for Bacilli dysenteriae. Positive bacteriophage was present. Complete lysis in four hours.

One week later stool examination was negative for dysentery bacilli and negative also for bacteriophage.

COMMENT

The effectiveness of specific bacteriophage therapy in this case is very striking.

The disappearance of Bacillus dysenteriae (Flexner) from the stool was rapid. The course of the infection was shortened and recovery was prompt.

The absence of a potent bacteriophage at the onset, and its presence for five days after the last oral dose was taken, is also significant.

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DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

MENINGITIS, Staphylococcus, Treatment With Specific Bacteriophage, Schelss, R. A. Am. J. Dis. Child. 44: 813, 1932.

A case is reported with recovery after intraspinal use of staphylococcus bacteriophage.

A review of the literature of staphylococcic meningitis is given, a classification of clinical types offered and a résumé of therapeutic measures and their efficacy made.

The literature of bacteriophage, to date, is covered, and evidence of the applicability of this agent to other staphylococcic infections is quoted.

A report on the first instance of the introduction of bacteriophage into the spinal canal is given, with comment on the resultant recovery in a true case of Staphylococcus aureus meningitis.

ERYTHROCYTES, Diameter of In Premature Infants, Van Creveld, S. Am. J. Dis. Child. 44: 701, 1932.

By means of the method of Price-Jones the changes in the size of the erythrocytes during the first months after birth were investigated in a large number of full-term and premature infants. One of the chief reasons for this study was the fact that directly after birth the blood is influenced by different factors which in themselves are able to change the size of the erythrocytes in a definite way. These factors do not act with the same intensity in full-term as in premature children. During the first two weeks after birth the Price-Jones curves of full-term and premature infants showed little difference. Whereas after that period the mean diameter in full-term children did not change very much, that in premature children decreased considerably; also the curve showed a different course from that in full-term children. The significance of the established differences and their relation to the newer conceptions of the cause of hemolysis after birth, to the anemia in premature children and to the physiologic loss of weight after birth are discussed in extenso.

The clinical significance of the anisocytosis and of the mean diameter of the erythrocytes in the analysis of anemias in the early months of life is explained by means of some examples (cases of icterus neonatorum gravis and congenital anemia).

BLOOD: Clinical Value of Uncorrected Color Index and of Cell Size in Pernicious Anemia, Goldhammer, S. M. Am. J. M. Sc. 184: 645, 1932.

The uncorrected color index represents the actual hemoglobin per cent reading (100 per cent as normal), over the percentage of red blood cells (5,000,000 being equivalent to 100 per cent), disregarding such factors as sex and the various hemoglobin standards.

Because of the wide range of normal red blood cell counts, 5,000,000 cells per c.mm. may be used as an average arbitrary standard. From 14 to 16 gm. of hemoglobin per 100 c.c. of blood are suggested as a standard of normal.

The average and serial color indexes in uncomplicated cases of pernicious anemia are above 1 before treatment; those of the male series are higher than those of the female.

Regardless of type of effective treatment, the influence on the color index is the same; the average and serial color indexes remain above 1 for about 6 weeks after treatment is started, then become less than 1.

In untreated and uncomplicated cases the lower the initial red blood cell count the higher the average color index. As the red blood cell count approaches the arbitrary normal following adequate treatment, the color index tends to approximate unity. Within the range of 4,000,000 to 5,000,000 red blood cells per c.mm. a level is reached at which the color index became 1 or less.

A single color index determination is not always diagnostic in cases of pernicious anemia, as individual readings may be above or below 1; but during relapse the average color index is always above 1 unless some complication is present.

The color index may be influenced by such complications as hemorrhage, chronic infections, glandular dystrophies and food deficiencies.

The increase in the percentage of red blood cells larger than 7.5 micra in early relapse, regardless of the severity of the anemia, may be used as a factor differentiating pernicious anemia from secondary anemia.

In untreated cases of pernicious anemia a high color index is always associated with a marked increase in the percentage of cells larger than 7.5 micra.

The presence of increased numbers of large cells in a blood film, regardless of the red blood cell count, is one of the earliest and most constant findings of the blood in beginning relapse in pernicious anemia.

RENAL FUNCTION, An Improved Concentration Test, Lashmet, F. H., and Newburgh, L. H. J. A. M. A. **99**: 1396, 1932.

The following instructions are furnished the patient:

Instructions for Renal Function Test

1. Date.....
   1. At 10 P.M. stop all fluid and food, except special diet until noon..... (38 hours).
2. Date.....
   1. At 8 A.M. empty bladder and discard urine
   2. Eat special diet only today
   3. Collect all urine from 8 A.M. today until 8 A.M..... in bottle 1. Finish specimen promptly at 8 A.M.
3. Date.....
   1. No breakfast today
   2. Collect urine at 10 A.M. in bottle 2
   3. Collect urine at 12 noon in bottle 3

Collect urine in dry bottles. Keep bottles in a cool place and keep well stoppered.

The special diet referred to is shown in the table:

Menu of the Diet

	Foods	Grams	Approximate Measure
Breakfast;	Corn flakes	15	½ cup
	Bread (toast)	60	2 slices, ⅜ inch
	Butter	20	2 squares or 1 level tablespoon
	Cream 40%	50	¼ cup
	Sugar	13	1 tablespoonful
Lunch;	Beef steak	100	4 by 4 by ½ inch
	Potato (baked)	80	1 about 2½ inches diameter
	Crackers	16	4
	Butter	20	2 squares, or 1 level tablespoon
	Dates	70	10
Dinner;	Potato (baked)	80	1 as above
	Lettuce	100	¼ head
	Crackers	16	4
	Butter	20	2 squares, or 1 level tablespoon
	Dates	30	4
	Peaches (canned—no juice)	85	1 half

In addition to foregoing, 1 gm. of sodium chloride (table salt) or ¼ level teaspoonful. No other salt is to be added to cooking or otherwise.

The diet above furnishes 700 gm. of available water in 24 hours and contains protein 40 gm., fat 104 gm., carbohydrate 204 gm. equivalent to 1900 calories. It contains 6.536 gm.

of nitrogen, 9.1 gm. of inorganic solids, 3.286 gm. of chlorine, and an excess of available base equivalent to 18 c.c. of normal solution.

The Test: All three specimens are tested quantitatively for albumin and their specific gravity recorded. For every 1 per cent of albumin 0.003 is subtracted from the observed specific gravity.

Results: Under definitely controlled conditions, normal kidneys are able to concentrate the urine to a specific gravity between 1.029 and 1.032, while diseased kidneys cannot. The lower the concentrating ability is, the greater the renal damage.

When determined under the conditions described, the specific gravity of the urine detects lowered kidney function before this fact is discernible by either the phenolsulphonphthalein test or determination of the blood nonprotein nitrogen.

Granulopenia and Agranulocytic Angina, Harkins, H. J. A. M. A. **99:** 1132, 1932.

Reports of thirty-six cases of recurrent granulopenia are collected from the literature.

Seventeen cases of granulopenia have been observed in the University of Chicago Hospitals. Eight of these seem to be primary granulopenia and in four more than one attack occurred.

The etiology of primary granulopenia is as follows:

(a) The oral lesions and sepsis are secondary to the granulopenia.

(b) The primary granulopenia may be due to some endogenic factor such as allergy, endocrine disturbance or congenital deficiency of the bone marrow.

(c) On the other hand, the causative factor may be an exogenic agent, acting either on a normal person or on a person with a special susceptibility. Unknown organisms, B. influenzae, and chemical poisons are to be considered.

(d) The causative agent acts chiefly on the bone marrow.

The following conclusions are made concerning the treatment of primary granulopenia:

(a) Oral antisepsis merely delays the entrance of malignant organisms into the system. Conversely, attempts to restore the white cell count to normal will not save the patient's life if sepsis is too advanced.

(b) Blood transfusion and roentgen rays are of no proved value.

(c) Chemical agents intended to stimulate the bone marrow are in general ineffective. Some evidence has collected to indicate that nucleotide is of value in primary granulopenia. Nucleotide did not affect the course of the granulopenia in acute benzene poisoning in rabbits.

BLOOD PLATELETS: A Method for The Simultaneous Enumeration of Reticulocytes and, Dameshek, W. Arch. Int. Med. **50:** 579, 1932.

This is an indirect method in which the diluting fluid is not only isotonic but contains brilliant cresyl blue for staining purposes and sodium citrate for use as an anticoagulant. This solution, which is a modification of the one devised by Buckman and Hallisey for counting the platelets directly, contains the following ingredients:

	gm. or c.c.
Brilliant cresyl blue	0.15
Sodium citrate	0.40
Sucrose	8.00
Water	100.00

The cane sugar and the sodium citrate are dissolved in distilled water to which is then added the brilliant cresyl blue. The resulting solution is mixed well and filtered. Three drops of a solution of formaldehyde (1:10) U. S. P., are added as a preservative.

This solution keeps well in a cold place, but it should be filtered every three or four weeks. It has the following advantages: (1) The stained platelets are easily seen. (2) The stained network and granules of the reticulocytes are well brought out. (3) The anticoagulant prevents clumping of the platelets. (4) The isotonicity of the solution aids in the proper separation and distribution of the blood cells.

Method.—One of the fingers is well cleaned with alcohol or acetone and then dried. A puncture wound is made. The first drop of blood is discarded. A fairly large drop (about 3 mm. in di-

ameter) of the staining solution is placed over the puncture wound, and the finger is gently squeezed so that a small amount of blood wells up into the drop of staining solution. The correct amount of solution to be placed on the finger and the proportion of blood to be squeezed into it can be learned only with experience. The proportion of blood to stain should be small (about 1:5) so that a well-spread preparation, not overcrowded with red blood cells, is obtained. The mixture of blood and stain is immediately transferred to a cover slip, which is then dropped on a slide. Cleanliness of glassware is essential.

The preparation is examined under oil immersion lens, preferably after from fifteen to forty-five minutes to permit complete staining of the platelets and reticulocytes. Counts may be made at any time within two hours. Counts made up to four hours, if the preparations are ringed with petrolatum, are also accurate. Permanent preparations cannot be made with this method. Blood platelets, even those measuring only 1 micron or less in diameter, are easily seen as highly refractile opalescent bodies taking a pale bluish stain. Reticulocytes are well stained, even the slightest degree of granulation and reticulation being easily seen. The rapid motion of some of the reticulocyte granules can easily be followed. White blood cells, which also take the stain, may easily be recognized and counted if desired.

One thousand red blood cells are counted (the microscopic field being cut down if desired by the insertion in the eyepiece of an appropriately perforated paper disk), and the number of platelets and reticulocytes seen during this enumeration is recorded. The number of reticulocytes is expressed as a percentage of the total number of red blood cells. The absolute number of platelets is obtained by: (1) performing a count of the red blood cells, and (2) solving the following equation, red blood count: 1,000: : platelets per cubic millimeter: platelets counted. This is most conveniently done by multiplying the first four figures of the red blood cell count by the number of platelets seen in counting 1,000 red blood cells. Thus, if in counting 1,000 red blood cells, 20 reticulocytes and 200 platelets are seen, and the red blood cell count is 3,000,000 per cubic millimeter, the percentage of reticulocytes is 2; and platelet count is 3,000 times 200, or 600,000 per cubic millimeter.

Normally, there is slight variation in the size and shape of the blood platelets.

The normal blood platelet count with this method in men ranges from 500,000 to 900,000 per cubic millimeter. The normal count in women is made uncertain by the complicating presence of the menstrual cycle. The intermenstrual range is from 400,000 to 800,000 per cubic millimeter.

Various procedures to check the accuracy of the method were undertaken. The experimental error is probably not greater than 70,000 per cubic millimeter when the erythocyte count is normal and not more than 10,000 per cubic millimeter when the erythrocyte and blood platelet counts are very low.

DIAZO REACTION: A Modified, in the Diagnosis of Typhoid Fever, Symons, P. H. S. A. Med. J. **6**: 594, 1932.

The method following is said to be more satisfactory and to give more reliable results than those heretofore used.

Stock Solutions.—A. Dissolve 0.9 gram of sulphanilic acid in water, add 9 c.c. of 37 per cent HCl, make volume up to 100 c.c. with distilled water.

B. Make up 5 grams of sodium nitrite to 100 c.c. with water.

C. A 1.1 per cent aqueous solution of pure anhydrous sodium carbonate or a 3 per cent solution of crystalline sodium carbonate is used as the alkali.

Preparation of Reagent.—In a beaker containing an ice-water mixture place an empty test-tube and a graduated cylinder containing 41 c.c. of distilled water.

Into the test tube pipette 1.5 c.c. of A and 1.5 c.c. of B, both cold; leave for five minutes, add 6 c.c. of B, again leave for five minutes, then pour the contents of the testtube into the water in the graduated cylinder, in which it has meantime been chilled by immersion in the ice-water mixture. Fifty cubic centimeters of reagent are thus obtained. The reagent is stable for at least twenty-four hours in the cold.

As the reagent decomposes on warming, evolving nitrogen, the evolution of a copious volume of gas while preparing it indicates that the temperature is too high and the reagent useless. The

reagent should also be quite colorless. When ice is not available, good results may often be obtained by substituting cold water, particularly if the solutions A and B be mixed slowly. The use of freezing mixtures might be considered.

The Test.—Mix 2 c.c. of the reagent with 5 c.c. of C. Within a minute add from a dropping pipette one drop of the urine to be tested, so that it falls slowly through the solution. Observe the color development of the drop of urine against a white background.

A positive reaction is shown by the immediate development of a red or orange-red color, which sometimes disappears very rapidly. Practically all urines give a yellow color which slowly intensifies; this may be neglected.

It was not considered worth while to estimate the strength of the reaction with quantitative accuracy, so that the following conventions were adopted.

One drop of urine dropped slowly through the reagent gives:

(1) A yellow color, intesifying slowly = negative (–).
(2) A brownish color = doubtful (?).
(3) A faint pink = doubtful (?).
(4) A definite pink = positive (+).
(5) Red = moderately strong positive (++).
(6) Deep red = strong positive (+++).

Urines for the test should be fresh and free from obvious bile; stale normal urines tend to give a brownish coloration, probably due to free ammonia. Production of color on mixing the reagent with solution C is usually due to a dirty tube.

B. PROTEUS: The Isolation of Organisms In The Presence Of, Fry, R. M. Brit. J. Exper. Med. **13**: 456, 1932.

The method depends on the fact that in the depths of a solid medium B. proteus grows in discrete colonies. The swab or other material from which a culture is to be made is spread on blood-agar, and melted agar at about 45° C. is then poured over the surface of the plate to a depth of 2 or 3 mm. and allowed to set. After incubation it will be found that any colonies of B. proteus growing between the two layers of agar show no tendency to spread, and colonies of other organisms can be easily picked out from among them. It generally happens, however, that some of the proteus spreads round the edge of the agar on to the upper surface, and this must be killed before any deep colonies are picked out. A satisfactory method is by flooding the surface of the plate with saturated mercuric chloride solution for about thirty seconds. This is washed off with tap-water, and some of the surface growth of proteus is scraped off with the end of a microscope slide to enable the deep colonies to be examined. These can then be picked out and subcultured.

If the colonies are very crowded, and it is thought that the one picked out may be contaminated by a neighboring colony of B. proteus, it is advisable to cover the subculture with a layer of agar in the same way. By this means, repeated more than once if necessary, it is possible to get a pure culture of most organisms, no matter how thick the contamination may be.

TISSUE STAIN: Mallory's Connective Tissue Stain Following Hematoxylin, Weiss, S. Stain Techn. **8**: 131, 1932.

Slightly overstain in Mayer's hematoxylin (50 gm. potassium alum and 0.2 gm. sodium iodate added to 1 liter 0.1 per cent aqueous hematoxylin). Wash; and stain 30 seconds to 1 minute in 0.04 per cent aqueous acid fuchsin. Stain 4 minutes in: 0.5 gm. anilin blue and 2 gm. orange G dissolved in 100 c.c. of 1 per cent aqueous phosphomolybdic acid. Pass through 95 per cent alcohol to absolute; clear in xylol and mount in balsam.

TYPHOID FEVER, H and O Agglutination As An Aid In The Diagnosis Of, Dulaney, A. D., Wilkle, W. T., and Trigg, R. Am. J. Pub. Health, **22**: 1033, 1932.

From a study of 161 serums from 41 cases of typhoid, 30 cases of nontyphoid febrile diseases, and 90 vaccinated (antityphoid) individuals, the H and O titers being determined in each case, the authors conclude that:

1. Both H and O agglutinins are produced in typhoid fever.
2. O agglutination in high titers (1-500 or greater) is very suggestive of typhoid infection.
3. Lower titers do not rule out O agglutination due to related infections or vaccination.

AMEBIASIS, Craig Complement Fixation Test For In Chronic Ulcerative Colitis, Kiefer, E. D. Am. J. M. Sc. **183**: 624, 1932.

That it is extremely difficult to differentiate between chronic ulcerative colitis and chronic amebic colitis has been pointed out and discussed.

The result of the Craig complement-fixation test for infection with Endamoeba histolytica when applied to patients with chronic ulcerative colitis is reported showing that 15 out of 19 cases gave a strongly positive reaction.

No conclusions as to the significance of this finding are drawn, but it is suggested that chronic ulcerative colitis may be a pyogenic infection of the colon superimposed upon an original amebic ulceration.

Abstracts of the case histories of the 19 cases tested are reported.

MELANOMA: Simple Technique for the Dopa Reaction, Laidlow, G. F., and Blackberg, S. L. Am. J. Path. **8**: 491, 1932.

The reagents required are a stock solution of dopa and the Sorensen buffers.

The Stock Dopa Solution is a 1:1000 solution of 3,4-dioxyphenylalanin (abbreviated to dopa) in distilled water. (When ordering, specify "for Bloch's dopa reaction." Supplied by the American branch of Hoffmann-LaRoche, Nutley, New Jersey, at 95 cents per gram. With the minute quantity used, the cost of staining a dozen or more sections is less than 2 cents.)

Dissolve 0.3 gm. of dopa powder in 300 c.c. of cold distilled water. Keep well corked in the refrigerator, where it will remain good for many weeks. The solution is usable as long as it is colorless or only slightly tinged with red. Darker red solutions should be rejected; they oxidize too quickly and overstain the sections.

THE BUFFERS

Dissolve 11 gm. of disodium hydrogen phosphate (Na_2HPO_4 plus $2H_2O$) in 1000 c.c. of distilled water.

Dissolve 9 gm. of potassium diphydrogen phosphate (KH_2PO_4) in 1000 c.c. of distilled water. Both of these buffers are kept in the refrigerator.

Just before cutting the sections, buffer to 7.4 by adding 2 c.c. of the potassium phosphate and 6 c.c. of sodium phosphate buffer to 25 c.c. of the stock dopa solution. For a small batch of a dozen sections we use 15 c.c. of the buffered solution, but we prepare double the quantity immediately required in order to have enough to renew solution in half an hour. Return the stock dopa solution and the surplus of the buffered solution to the refrigerator immediately; at room temperature the stock solution soon oxidizes and turns red, the buffered solution tends to turn brown.

At a given temperature, the speed of the reaction is determined by the P_H. At 7.4 the reaction will be finished in four or five hours at 37° C.

TEMPERATURE AND TIME

The dish of dopa containing the sections is put in the incubator at 37° C. for about half an hour. Then the fluid is replaced by fresh solution, which in the meantime has been kept cold in the refrigerator. For this renewal of the solution there is a reason. Some tissues are sufficiently acid to lower the P_H below 7.0, in which event the fluid remains red and the cells do not oxidize dopa to melanin. They remain colorless. On the other hand, tissue that has been in formalin, especially neutralized formalin, hastens the oxidation, darkens the fluid prematurely and easily overstains. Since it is impossible to foresee the presence of these disturbing factors, and since water cannot be used to wash them out, it is a routine practice to change the dopa. The first dopa washes out any objectionable substances and the reaction proceeds unhindered in the fresh solution. At times sections of the rectum and colon are so acid that two changes of dopa are required before the red of the acid solution changed to sepia brown of a correct reaction. Under these circumstances a liberal quantity of dopa solution should be used.

Having replaced the first dopa with fresh solution, the reaction is inspected every half hour. In two or three hours the fluid turns reddish, then sepia brown. The appearance of the sepia tint signals the end of the reaction. At this point a section is rinsed and examined under the microscope.

REVIEWS

Books and Monographs for Review should be sent direct to the Editor, Dr. Warren T. Vaughan, Professional Building, Richmond, Va.

The Cardiac Output of Man in Health and Disease*

AN inclusive review of historical investigations, a detailed presentation of the author's acetylene method of determination, and a comprehensive discussion of the clinical and experimental significance of this method of study. For further remarks see editorial in this number.

Physiological Chemistry**

MATHEWS' Physiological Chemistry is so well known as a standard textbook that it requires no introduction. A volume which retains this distinction for fifteen years and passes through five editions must necessarily both fill the need for which it was written and avoid the extremes and superlatives that occasionally detract from the value of a book written by one who is an enthusiast in some particular branch of his chosen field.

There has been no significant change in the arrangement or manner of presentation in the latest edition, although the recent advances in the field of physiological chemistry have been incorporated and the volume is entirely up to date.

A System of Clinical Medicine†

THIS is not so much a textbook of medicine as a treatise on physical diagnosis and differential diagnosis, combined. It opens with general instructions on the taking of a history and the making of a physical examination and then takes up the various regions of the body, classifying the illnesses appertaining thereto on the basis primarily of symptoms. Following a discussion of symptoms there is a detailed discussion of physical signs and any laboratory procedures that may be indicated, and then a presentation of differential diagnosis. Prognosis and treatment are lightly touched upon.

The fact that this volume has gone through eight editions indicates its worth. It would find its chief usefulness with students of medicine and with general practitioners who have but few of the more highly technical volumes to refer to.

The Clinical Interpretation of Blood Examinations‡

THE intention of this volume is indicated sufficiently in its title. The clinician or the general physician having received a brief report from the laboratory concerning some particular test, what is to be the correct interpretation as regards this particular patient?

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*The Cardiac Output of Man in Health and Disease. By Arthur Grollman, PH.D., M.D., Associate Professor of Physiology in the Medical School of the Johns Hopkins University. Cloth. Pages 325. Springfield, Ill. Charles C. Thomas, 1932.

**Physiological Chemistry. A Textbook and Manual for Students. By Albert P. Mathews, Ph.D., Carnegie Professor of Biochemistry, The University of Cincinnati. Fifth Edition. Illustrated. Cloth. Pages 1233. William Wood & Company, New York, 1930.

†A System of Clinical Medicine. Dealing With the Diagnosis, Prognosis, and Treatment of Disease for Students and Practitioners. By Thomas Dixon Savill, M.D. Lond. Eighth Edition. Cloth. Pages 1019. William Wood & Company, New York, 1930.

‡The Clinical Interpretation of Blood Examinations. By Robert A. Kilduffe, A.B., A.M., M.D., F.A.S.C.P., Director, Laboratories, Atlantic City Hospital; Consulting Serologist, Betty Bacharach Home for Crippled Children, Atlantic City, N. J.; City Bacteriologist, Atlantic City, N. J.; Pathologist, Atlantic County Tuberculosis Hospital, Northfield, N. J.; Consulting Pathologist, Jewish Seaside Home, Atlantic City, N. J.; Etc. Octavo, 629 pages, illustrated. Cloth, Lea & Febiger, Philadelphia, 1932.

The present volume is the outgrowth of two smaller volumes by the same author, "The Clinical Interpretation of the Wassermann Reaction," and "The Clinical Interpretation of Blood Chemistry." Both of these smaller volumes found an immediate use and the author was stimulated to round out the subject by covering all phases of any form of examination of the blood that is done in the clinical laboratory. His effort has been most successful in its comprehensiveness and in its simplicity.

The literature has been very thoroughly covered and where opinions differ as to interpretation, these differences are recorded. Rarely used tests are included with those which are frequently employed and the most recent are also included. The volume will appeal to the internist equally as much as to the general practitioner.

Conquering Arthritis*

THIS volume, written for the layman, particularly the person suffering from arthritis, serves the purpose first of acquainting him with the nature of his malady and second, of enabling him to develop a healthy optimism concerning his disability. The work presents nothing particularly new from a research point of view but otherwise should be of interest not only to the layman with or without arthritis, but also to the physician who has made no special study of the disease but who wishes to inform himself on the most recent developments in classification and treatment.

The Doctor and His Investments†

THIS is really a worth while book. One has not read far into it before one becomes convinced of the sincerity of the writer and of the fact that the book is in no sense a stock promotion scheme. Mr. Rukeyser has contributed widely to The Journal of the American Medical Association's medical economics, Dental Survey, several of the prominent New York papers and is associate in journalism at Columbia. The type of advice that he gives is sound, if only one would follow it when it actually comes to making investments. Possibly, for the younger physician, the more important part of the volume is the negative part, that is, a discussion of who is economically not in a position to invest in stock. The writer emphasizes especially that one should cover his future by adequate life insurance before considering any other investment. He is not at all certain that home owning is a good thing unless one has a large family. He is still more dubious about owning stock in medical art buildings. The discussion of stocks and bonds is very much along the same line that one would find in any other introductory discussion of the subject, but throughout the author applies the facts to the needs of the physician.

The volume can be most highly recommended.

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*Conquering Arthritis. By H. M. Margolis, M.D. Cloth. Pages 192. The Macmillan Company, New York, 1931.

†The Doctor and His Investments. Financial Policy and Technique for the Physician. By Merryle Stanley Rukeyser, B.Lit., M.A., Financial Editor, Medical Economics and Dental Survey. Cloth. Pages 330. P. Blakiston's Son & Co., Inc., Philadelphia.

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EDITORIAL

Cardiac Output as a Functional Test

THE popularization of studies of functional pathology has resulted in the elaboration of very adequate methods for the study of the functional capacity of the kidneys, the thyroid gland, the internal secretion of the pancreas, the stomach and to a certain extent the liver. Progress has not been as rapid in the development of methods for study of circulatory function. Attempts have been made to assay the function of the circulatory and respiratory systems by vital capacity studies such as those elaborated by Dreyer[1] and by Peabody and Wentworth,[2] or the elaborate system devised by Frost,[3] and by study of the response of the pulse and blood pressure to exertion, and the breath holding, expiratory force, and expiratory fatigue test of Flack,[4] but all of these have been found to have only limited value, the conclusion usually being reached that the patient's subjective symptoms still remain the best measure of circulatory efficiency. And yet it is

at once obvious that such criteria will show evidence of functional impairment only after such impairment has progressed to a relatively pronounced degree.

Much can be learned of the conduction function within the heart by the electrocardiogram, but the need of a more direct and definite measure of cardiac function has been obvious. Methods of estimating the output of the heart have been devised and improved over years until they have been so simplified that we may look toward the perfection of this test to a stage where it can be used simply and easily as an office routine. If, as stated by Grollman, the cardiac output per minute, ratioed to body surface area, is a constant, it is readily understandable that this ratio, termed the "cardiac index," may assume a clinical value comparable to that of the metabolic rate determination.

Early studies of cardiac output were necessarily crude and were made by such methods as measuring the size of the ventricles in the cadaver, measuring output in lower animals and calculating the probable proportionate output in man, measuring the rate and volume of blood flow in an artery and calculating therefrom that in the aorta, measuring the appearance time of potassium ferrocyanide in the vein of one extremity after its injection into the vein of the opposite extremity, plethysmographic studies, and other ingenious indirect methods.[5]

Fick in 1870 elaborated a concept which has since been called the Fick Principle, which with adaptations, modifications and improvements has been the basis of most of the newer methods and simplifications of this study.

One can determine the amount of oxygen consumed by a body per minute time (as in the familiar metabolism test). One can determine the volume of oxygen in 100 c.c. of arterial blood and that in 100 c.c. of mixed venous blood by direct measurement. The difference between these two latter figures represents the amount of oxygen given up to the tissues by each 100 c.c. of blood. The total oxygen consumption per minute divided by the number of volumes given up to the tissues by each 100 c.c. of blood indicates the number of 100 c.c. volumes of blood which must have passed through the heart and been distributed to the tissues per minute time. Fick also pointed out that the same information could be obtained by knowledge of the carbon dioxide content of arterial and venous blood and the carbon dioxide output per unit time through the lungs. If arterial blood contains 18 volumes per 100 c.c. of oxygen and venous blood from the right side of the heart contains 12 volumes, then every 100 c.c. of blood gave up 6 volumes of oxygen to the tissues. If the total oxygen consumption of the individual is 60 c.c. per minute, it is obvious that ten 100 c.c. volumes each giving up 6 c.c. are necessary to distribute the 60 c.c. to the tissues in one minute's time. The cardiac output per minute is then 1000 c.c.

Obviously, the necessity of obtaining blood by puncture of an artery and directly from the right ventricle or from the vena cava prevent clinical application of this method. Gradually other methods have been elaborated based upon the Fick Principle, first using inert gases such as nitrogen and later, foreign gases. The method recommended by Grollman[6] involves the use of acetylene gas.

If a gas is in contact with a liquid it will become dissolved in and diffused through that liquid. The greater the concentration of the gas above the liquid the more will be dissolved in the liquid. The greater the volume of liquid the

less will be dissolved per unit volume. If one is breathing acetylene gas, this gas is in contact with a liquid (blood) in the lungs. If one knows the concentration of the acetylene gas in the air of the lung and its concentration in the blood per unit volume (in equilibrium with the acetylene in the air of the lungs) and if one knows the solubility of the gas in blood, one can compute the total volume of blood required to make that concentration in the blood. The tension and rate of absorption of acetylene are determined and from this the volume of blood flowing through the lungs per unit time is calculated. This of course corresponds with the volume flowing through the heart per unit time except in certain rare congenital anomalies.

It is not necessary to determine the concentration of acetylene in the blood. The volume of liquid in a gas-liquid system regulates the rate of absorption. If one knows the solubility of the gas in the liquid and its rate of absorption one can compute the volume of the liquid. In the case under consideration the volume of the liquid is the amount of blood passing through the lungs per unit time. One liter of blood at body temperature dissolves 740 c.c. of acetylene when the tension of this gas is 760 millimeters mercury. In the test, therefore, the patient rebreathes in a closed lung-bag system, a mixture of oxygen and acetylene. The acetylene concentration in this closed system is determined at the beginning and end of the experiment. The difference in these two concentrations divided by the time gives the rate of absorption. The solubility being known, the volume of fluid to which the gas was exposed may be calculated, and this is the cardiac output per unit time. Since the experiment involves respiratory activities which modify the normal circulation, certain corrections are made on the basis of simultaneous determinations of oxygen consumption in the lung-bag system and the determination of oxygen consumption per unit time as in the ordinary metabolism test.

Thus we see that a calculation of the cardiac output can be done simply, by methods of gas analysis, with certain corrections analogous to the corrections in the determination of oxygen consumption in metabolism tests. If, at rest, or under basal conditions the cardiac output is a constant, proportional to the surface area (the cardiac index), a method is at once available for study of the cardiac output in health and in the various diseases. The cardiac output of normal individuals per square meter of body surface is 2.2 plus or minus 0.3 liters per minute.

Grollman devotes considerable attention in his monograph to the results of clinical studies of cardiac output and to its possible clinical significance. For example, the intake of large amounts of fluid increases the cardiac output, thereby entailing more work on the part of the heart. In a heart already damaged by myocarditis this increased strain might work disadvantageously in certain cases of fever in which the usual procedure is the forcing of fluid. A knowledge of the cardiac output is of distinct importance in diseases of the heart and circulation but often desirable also in conditions in which the circulation is only secondarily affected. The moot question concerning the desirability of routine digitalization in pneumonia, previously discussed in these columns,[5] may receive further elucidation by this method of study. As shown by Grollman, by Harold J. Stew-

art and by others, a dilated heart when digitalized, returns to approximately normal size with a resultant increased cardiac output. A normal heart on the other hand when digitalized, contracts to less than normal size, with resultant decreased output. This would appear to be a potent argument against routine digitalization except in the presence of an obviously diseased, and especially a dilated heart.

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